Workshop in experimental design and applied statistics

Educational materials developed by Maegen Simmonds

Agricultural Field Statistics Package App developed by Ian King Kyle, Jason Moore, and Maegen Simmonds

Funded by USAID

Group introductions

Who are the instructors?

- Mark Bell, Professor, University of California, Davis, Email: mark.andrew.bell@gmail.com
- Miguel Macias, University of California, Davis, Email: mmaciasgonzalez@ucdavis.edu

Workshop Goals

- To understand experimental design and principles of statistical analysis to produce trustworthy results and interpretation.
- To learn how to use the free Agricultural Field Statistics Package computer app to analyze and visualize your data

Workshop Goals

- What do YOU want to learn?
- Write down 3 of your goals and we will create a list of your top priorities for the whole group

What are we doing today?

- Instruction 9:00 – 11:00 AM
  - Introductions
  - Section 1: Experimental design, ANOVA, hypothesis testing
- Tea break 11:00 – 11:30 AM
- Instruction 11:30 – 1:00 PM
  - Section 2: Agricultural Field Statistics Package app
  - ANOVA and interpretation with 1 treatment and with 2 treatments, factorial
- Lunch 1:00 – 2:00 PM
- Instruction 2:00 – 2:30 PM
- Tea break 2:30 – 3:00 PM
- Instruction 3:00 - 4:00 PM

What are basic experimental designs do you use in your research?

Examples:

1. Complete Randomized Design
2. Randomized Complete Block Design
3. Split-plot Design

Note: “Factorial” is a treatment structure that can occur within any of the experimental designs. We will also cover this.

Section 1 Learning Objectives

Have basic understanding of:

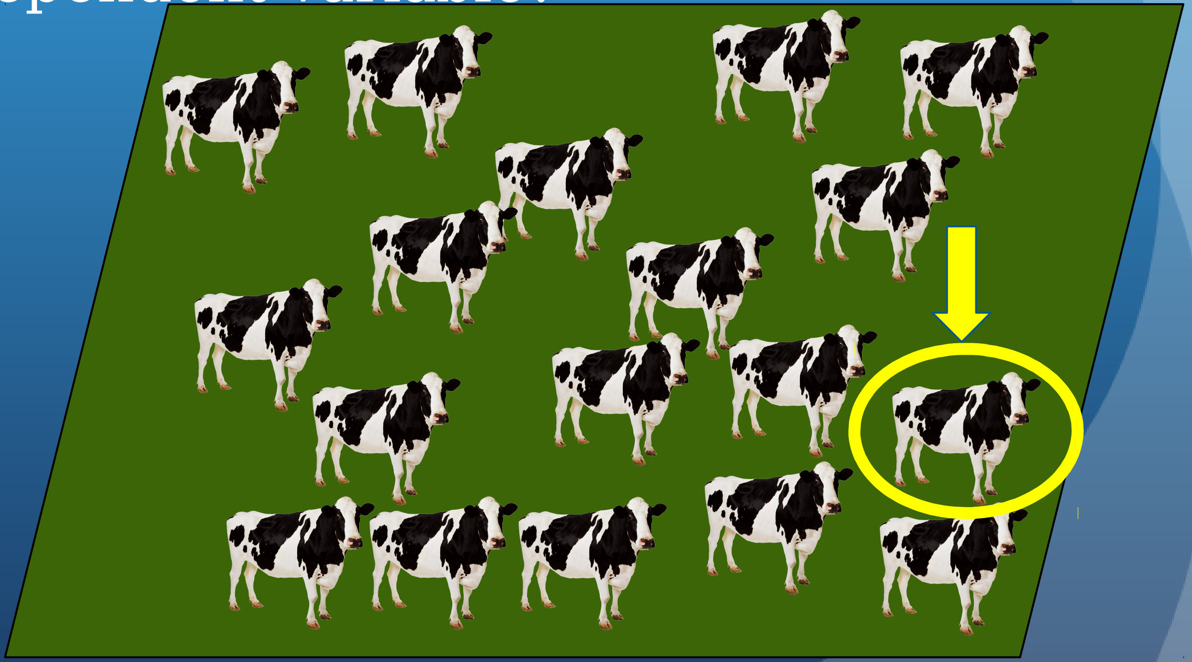
1. Elements of an experiment
2. Complete Randomized Design (CRD)
3. Hypothesis testing
4. Statistical model for a CRD with one treatment
5. Principles of Analysis of Variance (ANOVA) for a CRD with one treatment
6. Elements in an ANOVA table for a CRD with one treatment

Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

What is the experimental unit? Cow

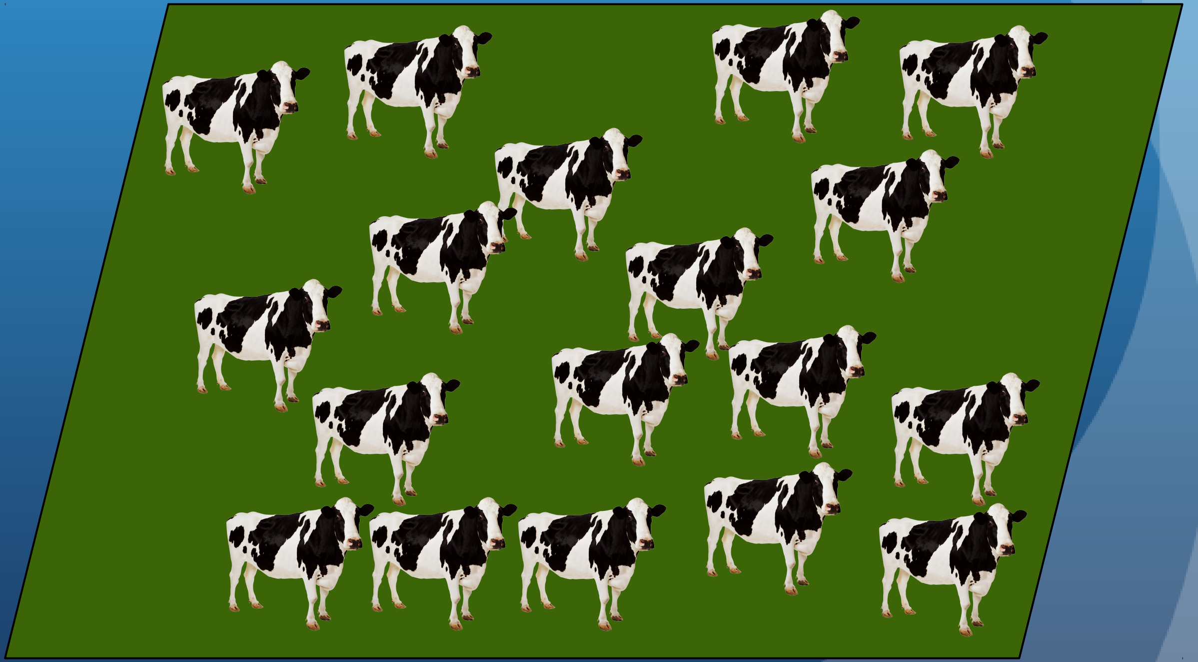
What is the dependent variable? Milk production performance

What is the independent variable? Milking practices



Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

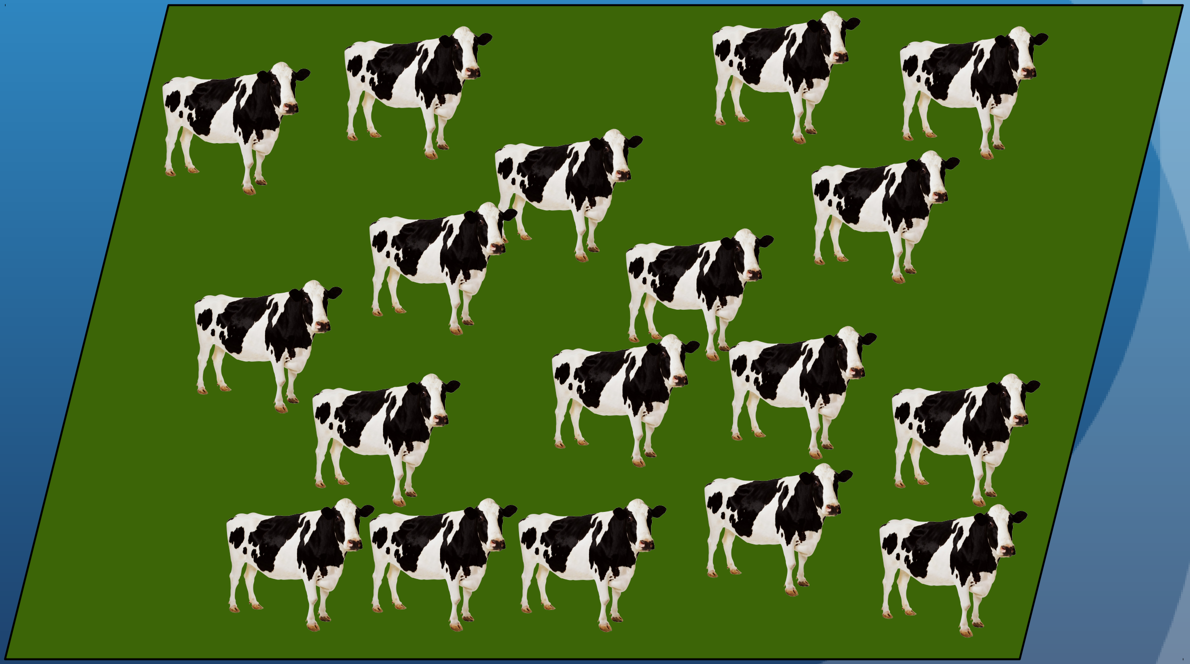
1. What experimental design would you use to determine if there is an effect of milking practice on milk production (kg)?



Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

1. What experimental design would you use to determine if there is an effect of milking practice on milk production (kg)?

If the experiment was at a *single* research station, using *cows with similar characteristics*, we may choose to apply the 4 treatments x 3 replications randomly to a subset of the cows.

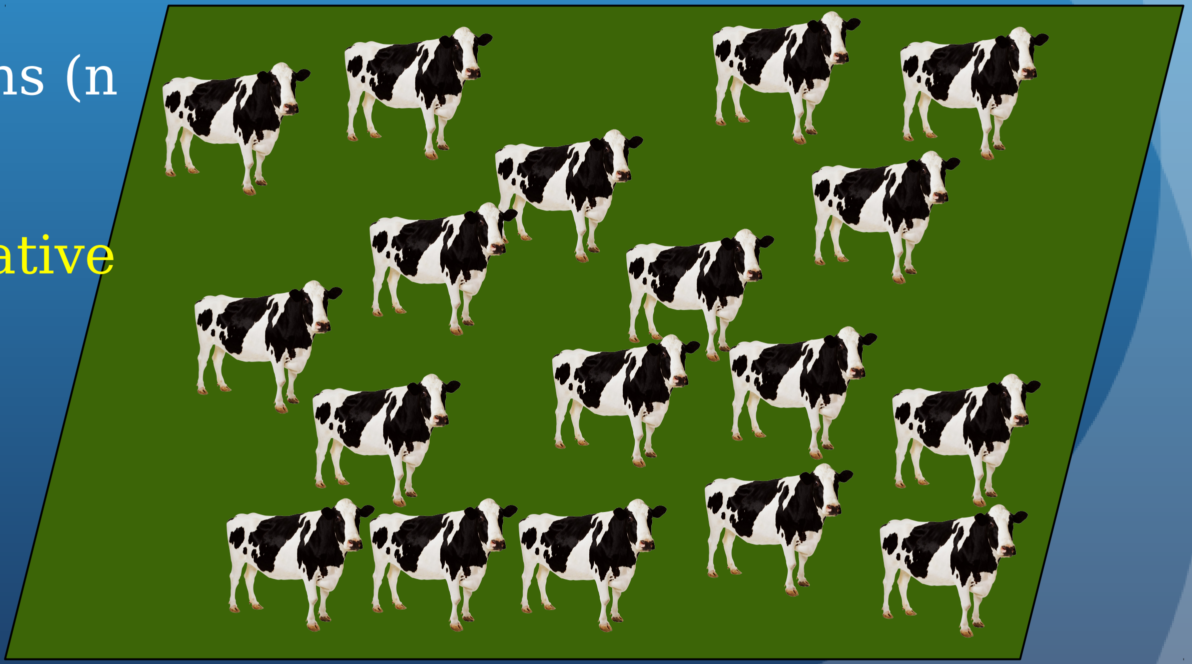


Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

This type of experimental design is called a Complete Randomized Design (CRD) (no blocking)

For 4 treatments with 3 replications (n = 12),

1. 12 representative cows are randomly selected,



Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

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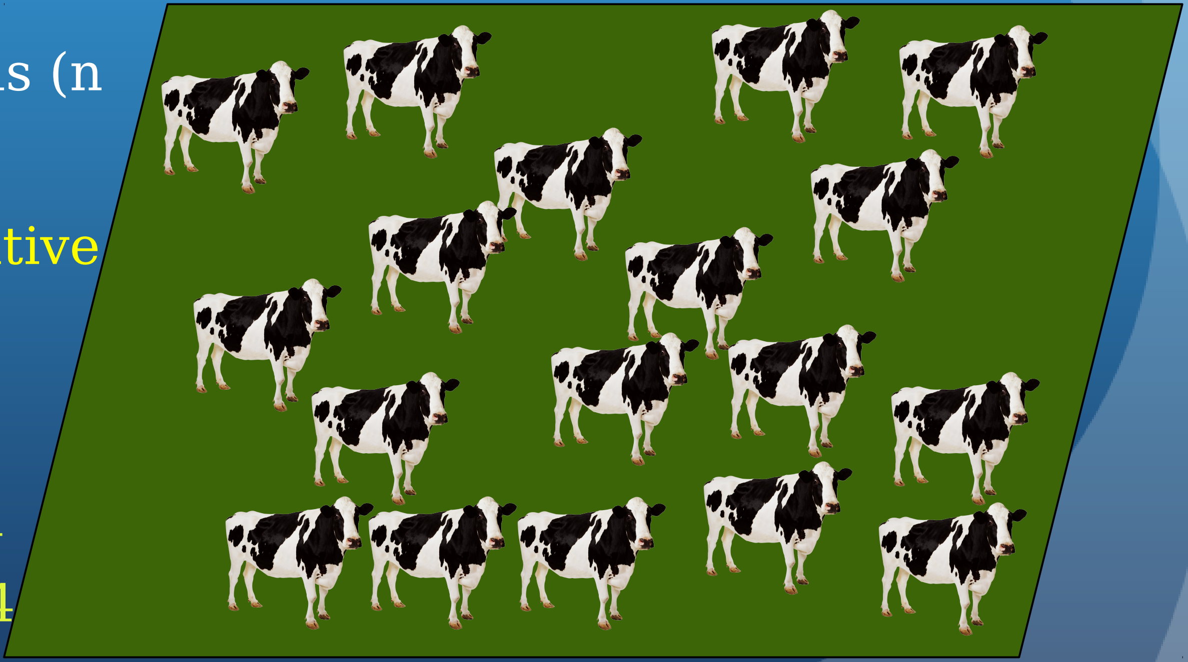
For 4 treatments with 3 replications (n = 12),

1. 12 representative cows are randomly selected,
2. And randomly assigned the 4 treatments.



Research question: Is there an effect of milking practice (hand-milk 2x/day, hand-milk 3x/day, machine-milk 2x/day, machine-milk 3x/day) on milk production performance in Sahiwal cows?

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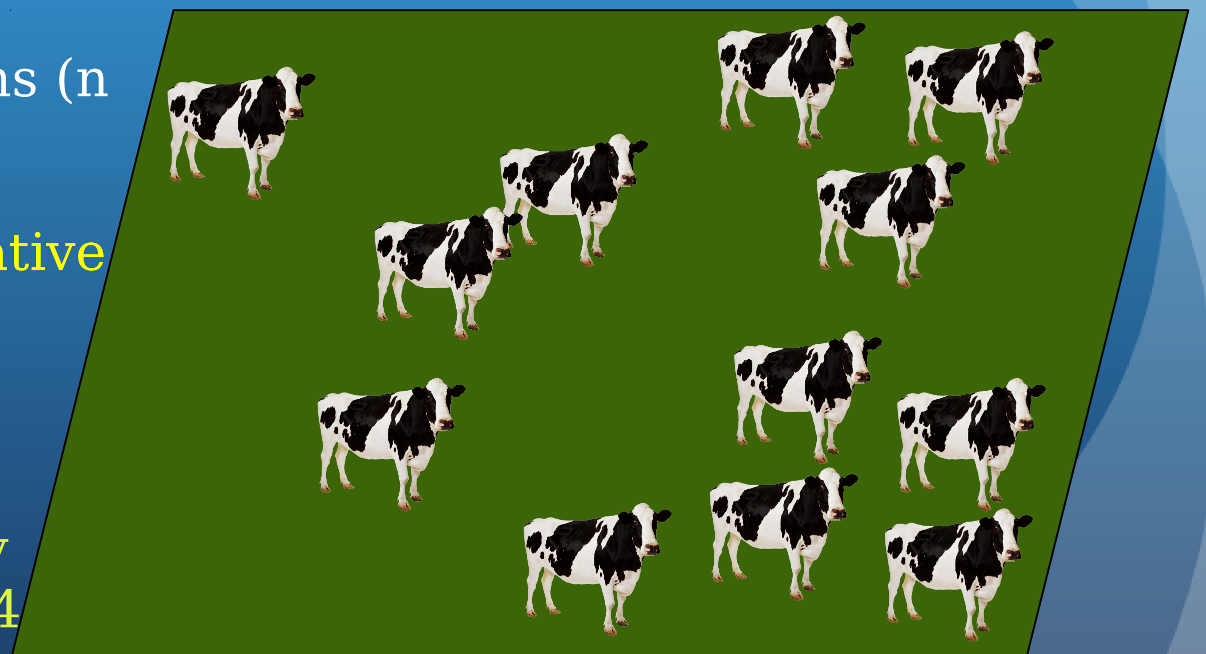
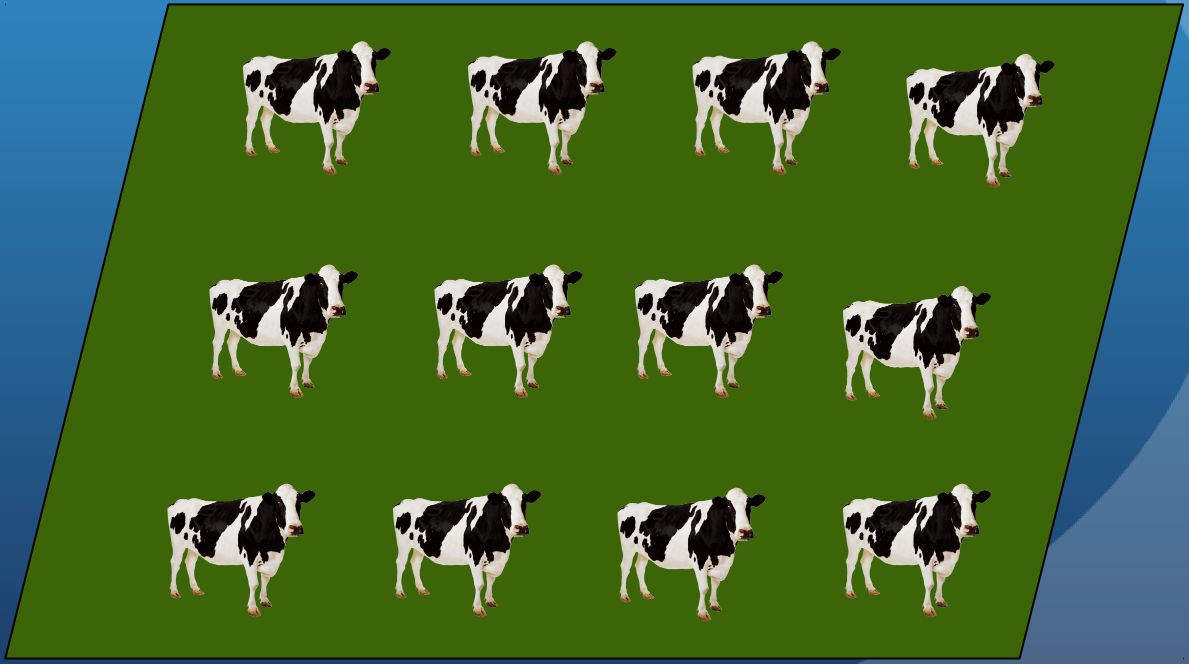
2. And randomly assigned the 4 treatments.



Replications versus subsamples: important reminders

1. What is your experimental unit?
2. Do you collect a sample from it more than once? If so, your data are subsamples. If not, they are replications.

For example, if you measured the milk production of each cow (experimental unit) more than once, would each observation be a replication or subsample?



Replications versus subsamples: important reminders

1. What is your experimental unit?

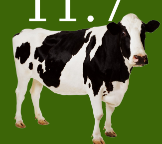
2. Do you collect a sample from it more than once? If so, your data are subsamples. If not, they are replications.

3. If your data consists of subsamples, you must *average* the subsamples for each experimental unit *before* loading data into the app. Otherwise, your data analysis will not be correct.

Let’s collect the milk production data (dependent variable)!

Observed milk production (kg)

12 cows and 12 total observations (n = 12). Each observation (Y_{ij}) identified by i^{th} treatment and j^{th} replication.

	(1) Hand-milk 2x	(2) Hand-milk 3x	(3) Machine-milk 2x	(4) Machine-milk 3x
(1)	8.6 	11.2 	9.6 	11.5 
(2)	9.3 	10.8 	9.3 	12.0 
(3)	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Observed milk production

Are these data replications or subsamples?

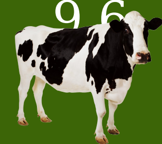
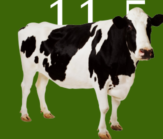
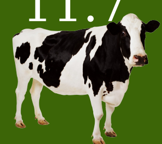
12 cows and … ons (n = 12). Each observation (Y_{ij} … d by i^{th} treatment and j^{th} replication.

	(1) Hand-milk 2x	(2) Hand-milk 3x	(3) Machine-milk 2x	(4) Machine-milk 3x
(1)	8.6 	11.2 	9.6 	11.5 
(2)	9.3 	10.8 	9.3 	12.0 
(3)	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Observed milk production

What was the milk production for $Y_{3,1}$?

12 cows and … ons (n = 12). Each observation (Y_{ij} … d by i^{th} treatment and j^{th} replication.

Replication	(1) Hand-milk 2x	(2) Hand-milk 3x	(3) Machine-milk 2x	(4) Machine-milk 3x
(1)	8.6 	11.2 	9.6 	11.5 
(2)	9.3 	10.8 	9.3 	12.0 
(3)	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Research question: Is there an effect of milking practice on milk production performance in Sahiwal cows?

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Overall mean: 9.3

In other words, does mean milk production differ among milking practice treatments?

In statistics, we actually test the *null* hypothesis (H_0).

Which is what?

All treatment means are equal

$$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$$

We are actually interested in the alternative hypothesis (H_A), which is what?

$$H_A: \textit{at least} \text{ two means differ}$$

What linear model predicts milk production for an individual cow in this experiment?

$$\mathbf{Y_{ij} = \ ?}$$

where i = treatment, and j = replication

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

$$\mathbf{Y_{ij} = \mu + \tau_i + \varepsilon_{ij}}$$

Y_{ij} is the dependent variable (what we are observing/measuring), which is equal to the grand mean (μ), plus the treatment effect (τ_i), plus random error (ε_{ij}).

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

In other words, each observation deviates from the mean of all the samples due to the **effect of the treatment** and due to unexplained **error**.

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

What things can cause error in an experiment?

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

Analysis of Variance (ANOVA) tests the validity of your statistical model using an F-test. The F-test determines if the treatment variance is sufficiently larger than the *within* variance.

If it is, we reject H_0 that all means are equal. If it's not, we fail to reject H_0.

ANOVA partitions the total variation between each observations and the mean (Total Sums of Squares) into the variation due to each factor in the statistical model.

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

Let’s fill in the ANOVA table together for the milking practices example, and then use the AIP App to do it for us.

ANOVA table

Response (Y_{ij}) = milk production

Source of variatio n	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**

First, we have the total variation among all samples.

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total					

The TOTAL variation is due to variation due the treatment (milking practice) and random error.

ANOVA table

Response (Y_{ij}) = milk production

Source of variatio n	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total					

The TOTAL variation is due to variation due the treatment (milking practice) and random error.

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total					
Milking practice					
Error					

Next, let's calculate Total Sum of Squares (TSS).

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total					
Milking practice					
Error					

Total "Sums of squares" (TSS)

is the difference between each observation and the grand mean, squared, and all added up (i.e. sum of squared deviations).

$$TSS = \sum_{i=1}^{t}\sum_{j=1}^{r}(Y_{ij} - \bar{Y}_{..})^2$$

i = i^{th} treatment
j = j^{th} replication
t = total # treatments
r = total # reps

Grand mean (across all treatments and reps)

1st treatment, 1st rep

t^{th} treatment, r^{th} rep

$$TSS = \left(Y_{1,1} - \bar{Y}_{..}\right)^2 + \left(Y_{1,2} - \bar{Y}_{..}\right)^2 + \ldots + \left(Y_{t,r} - \bar{Y}_{..}\right)^2$$

$TSS=\sum_{i=1}^{t}\sum_{j=1}^{r}(Y_{ij}-\bar{Y}_{..})^2$ Total Sums of Squares

1st treatment, 1st rep — Grand mean — 4th treatment, 3rd rep

$$TSS=(8.6-9.3)^2+(9.3-9.3)^2+\ldots+(11.8-9.3)^2=\boxed{18.1}$$

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total		18.1			
Milking practice					
Error					

Degrees of freedom (df) = total number of independent observations

An easy way to remember:

Df is the total # of elements used to estimate the parameter(s) of interest, minus total # of parameters.

- df = e - p

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total		18.1			
Milking practice					
Error					

“Within” variation of 12 elements relative to 1 estimated parameter, the grand mean

Total Sums of Squares:

$$TSS = \sum_{i=1}^{t}\sum_{j=1}^{r}(Y_{ij} - \bar{Y}_{..})^2$$

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

Thus, total df = 12 – 1 = 11

Grand mean was estimated based on 12 individuals

$$TSS = \sum_{i=1}^{t} \sum_{j=1}^{r} (Y_{ij} - \bar{Y}_{..})^2$$

i = i^{th} treatment
j = j^{th} replication
t = total # treatments
r = total # reps

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice					
Error					

Recall, TOTAL variation is due to variation due the treatment (milking practice) and random error.

Thus, Total SS = Treatment SS + Residual SS

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	$\boxed{18.1}$			
Milking practice					
Error					

But how do we calculate Treatment SS and Residual SS?

Total SS = SS Treatment + SS Residual

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice					
Error					

Let’s start calculating SS of Treatment.

Total SS = SS Treatment + SS Residual

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice					
Error					

Sums of squares of treatment (SST) is the difference between each treatment mean and the grand mean, squared, all added up, and multiplied by the total number of reps.

$$SST = r\sum_{i=1}^{t}\left(\overline{Y}_{i.} - \overline{Y}_{..}\right)^2$$

i = i^{th} treatment
t = *total # treatments*
r = *total # reps*

reps

1st treatment mean

Grand mean

t^{th} treatment mean

$$SST = r\left(\overline{Y}_{1.} - \overline{Y}_{..}\right)^2 + r\left(\overline{Y}_{2.} - \overline{Y}_{..}\right)^2 + \ldots + r\left(\overline{Y}_{t.} - \overline{Y}_{..}\right)^2$$

$$SST = r\sum_{i=1}^{t}\left(\overline{Y}_{i.} - \overline{Y}_{..}\right)^2$$

Sums of Squares of Treatment (SST): deviations due to milking practice

reps

1st treatment mean

Grand mean

$$SST = 3(9 - 9.3)^2 + 3(11.2 - 9.3)^2 + 3(9.3 - 9.3)^2 + 3(11.8 - 9.3)^2 = \boxed{17.1}$$

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice		$\boxed{17.1}$			
Error					

What are df for milking practice?

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice		17.1			
Error					

Treatment variance of 4 elements relative to 1 estimated parameter, the grand mean.

Sums of Squares of Treatment (SST)

$$SST = r\sum_{i=1}^{t}\left(\overline{Y}_{i.} - \overline{Y}_{..}\right)^2$$

i = i th treatment
t = total # treatments
r = total # reps

Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
8.6 	11.2 	9.6 	11.5 
9.3 	10.8 	9.3 	12.0 
9.1 	11.7 	9.0 	11.8 
9.0	11.2	9.3	11.8

Treatment means:

Grand mean = 9.3

Thus, total df = 4 – 1 = 3

Grand mean was estimated based on 4 elements

$$SST = r\sum_{i=1}^{t}\left(\overline{Y}_{i.} - \overline{Y}_{..}\right)^2$$

i = i^{th} treatment
t = *total # treatments*
r = *total # reps*

ANOVA table

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1			
Error					

Once we know df and SS of TOTAL & TREATMENT, we can solve for df error and SS error.

Total SS = SS Treatment + SS Error SS Error = Total SS – SS Treatment

Total df = df Treatment + df Error df Error = Total df – df Treatment

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1			
Error	8	1			

ANOVA table

Response (Y_{ij}) = milk production

What is causing the majority of the total variation in milk production?

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1			
Error	8	1			

The *majority* of total variation (TSS = 18.1) is due to milking practice (SST = 17.1)! But we don't know yet if the treatment effect is statistically significant.

ANOVA table

Response (Y_{ij}) = milk production

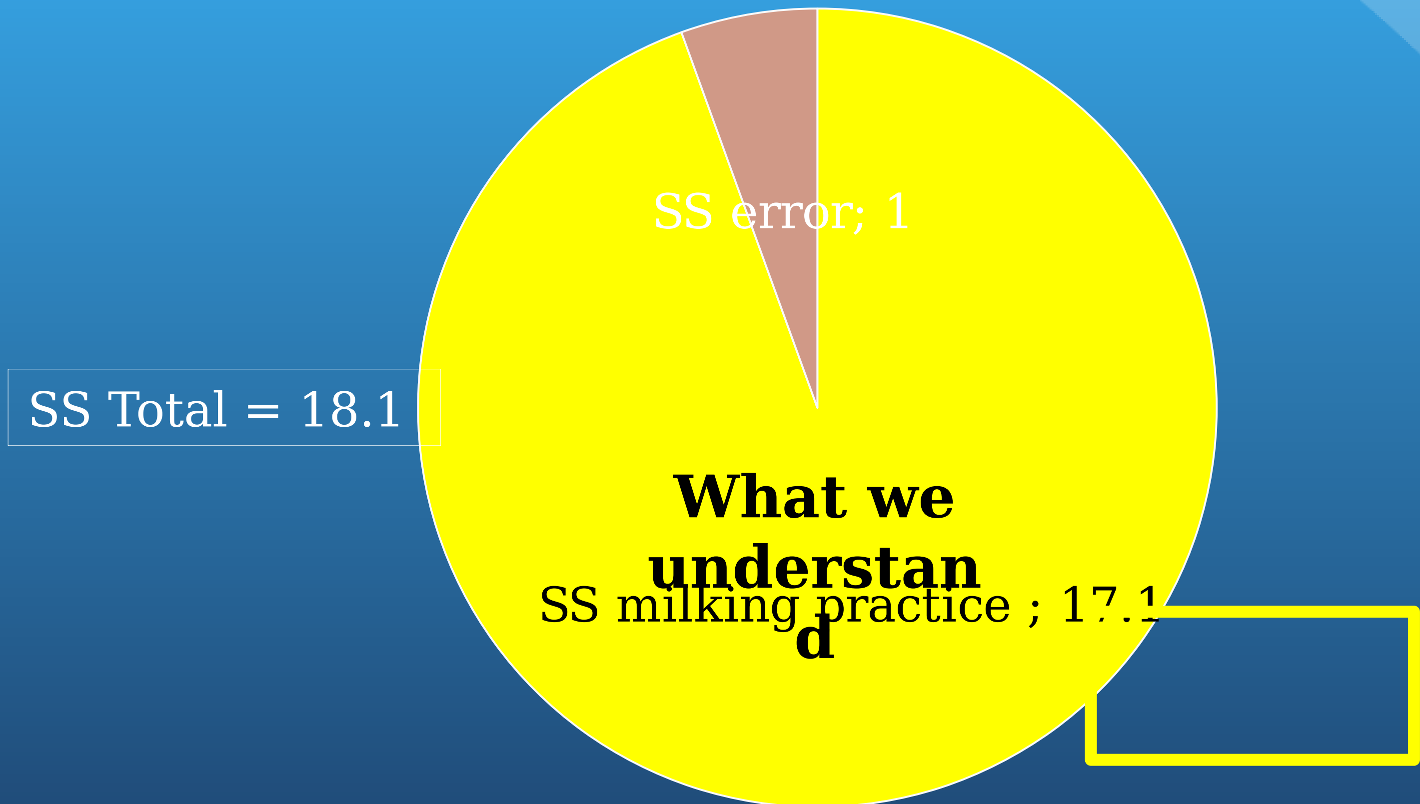
Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1			
Error	8	1			

SS Total = SS Treatment + SS Error

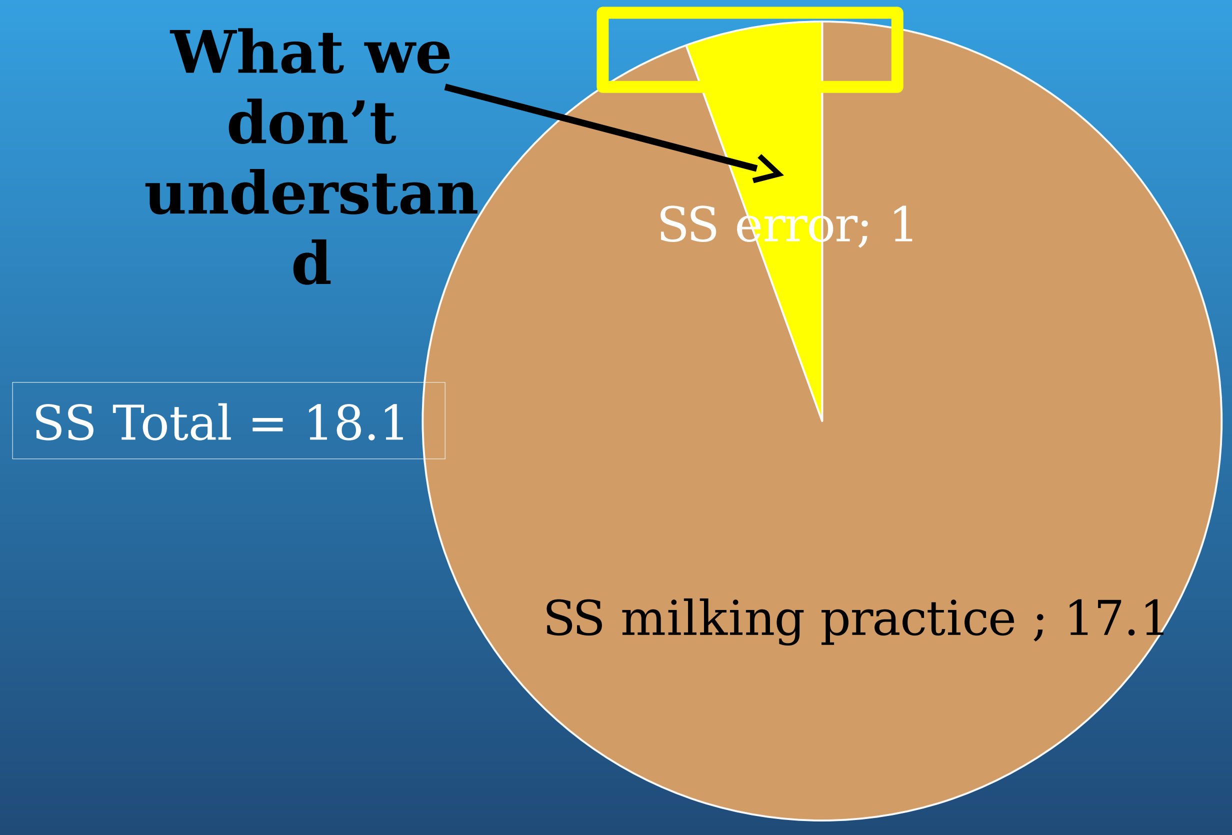
SS Total = 18.1

Total variation!

SS Total = SS Treatment + SS Error



SS Total = SS Treatment + SS Error



Next, calculate the mean squared deviations (MS) (Also known as sample variance) for milking practice and error.

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1			
Error	8	1			

Mean Squared Deviation (sample variance):

$$\boxed{s^2 = \frac{SS}{df}}$$

Mean Squared Treatment (MST) represents the t*reatment* variance,

$$\boxed{MeanSquaredTreatment = MST = \frac{SST}{df_t}}$$

while Mean Squared Error (MSE) is *"within"* sample variance

$$\boxed{MeanSquaredError = MSE = \frac{SSE}{df_e}}$$

Calculate the MS of treatment (MST):

$$MST = \frac{SST}{df_t} = \frac{17.1}{3} = 5.7$$

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1	5.7		
Error	8	1			

Calculate the MS of Error (MSE):

$$MSE = \frac{SSE}{df_e} = \frac{1}{8} = 0.125$$

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1	5.7		
Error	8	1	0.125		

"F test": H_0 = All treatment means are equal

Compares the probabilities associated with (1) F-calculated and (2) F-critical

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1	5.7		
Error	8	1	0.125		

“F test”: H_0 = All treatment means are equal

- F-calc: ratio of treatment sample variance (MST) to within sample variance (MSE)
- F-critical: the F-value associated your *chosen* acceptable level of probability of making a type I error (significance level). *We will use p=0.05

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1	5.7		
Error	8	1	0.125		

Treatment variance

F-calc

“within” variance

“F test”: H_0 = All treatment means are equal

1. Calculate F statistic: $$\text{F-calc} = \frac{\text{MST}}{\text{MSE}}$$

$$\text{which is really } \frac{\text{Treatment variance}}{\text{Within variance}}$$

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1	5.7		
Error	8	1	0.125		

Treatment variance

F-calc

“within” variance

“F test”: H_0 = All treatment means are equal

1. Calculate F statistic: $\text{F-calc} = \dfrac{\text{MS}T}{\text{MSE}} = \dfrac{5.7}{0.125} = 45.6$

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			
Milking practice	3	17.1	5.7	45.6	
Residual	8	1	0.125		

Treatment variance

F-calc

“within” variance

“F test”: H_0 = All treatment means are equal

1. Calculate F statistic: $$\text{F-calc} = \frac{\text{MS}T}{\text{MSE}} = \frac{5.7}{0.125} = 45.6$$
2. Determine P value

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1	5.7	$\boxed{45.6}$	
Residual	8	1	0.125		

Treatment variance

“within” variance

F-calc

“F test”: H_0 = All treatment means are equal

- P-value: probability of getting as extreme an F statistic by chance alone

Smaller the p-value, the more confident we are that the treatment effect is real (truth) and not a mistake (type I error)

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
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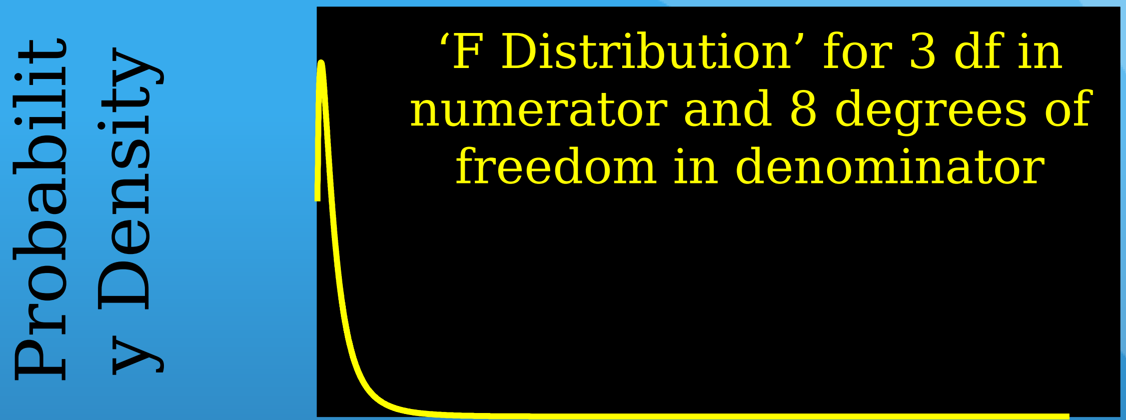
Error	8	1	0.125		

p-value

"F test": H_0 = All treatment means are equal



ANOVA table

Response (Y_{ij}) = milk production

Source of variatio n	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1			p-value
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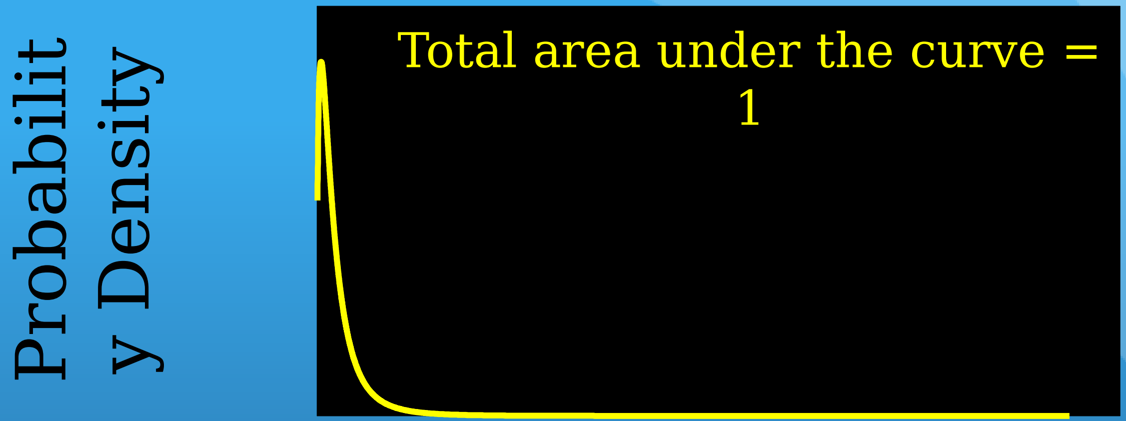

ANOVA table

Response (Y_{ij}) = milk production

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Total	11	18.1			p-value
Milking practice	3	17.1	5.7	45.6	
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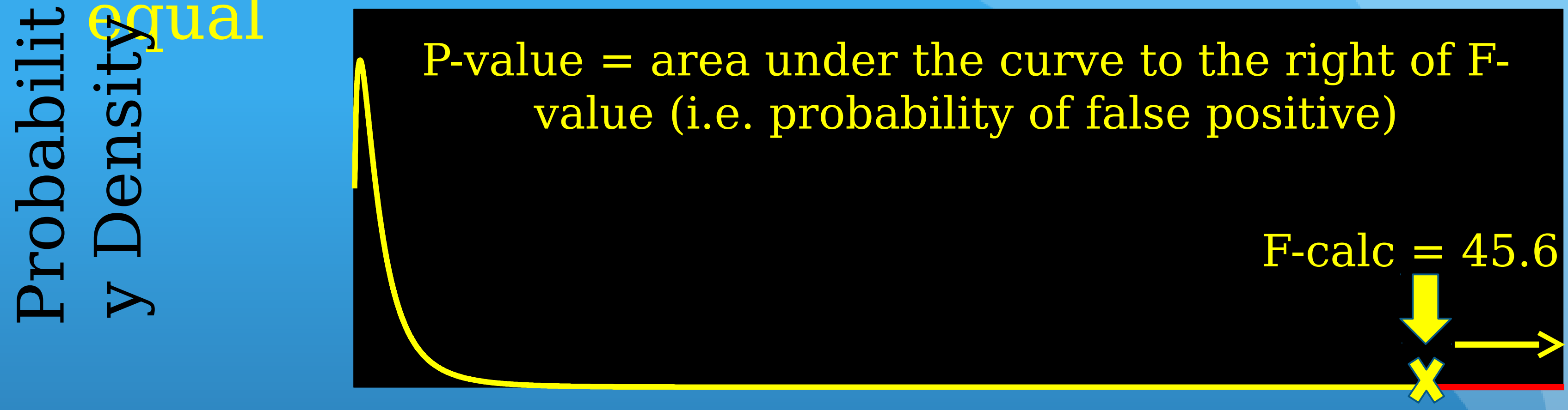

ANOVA table

Response (Y_{ij}) = milk production

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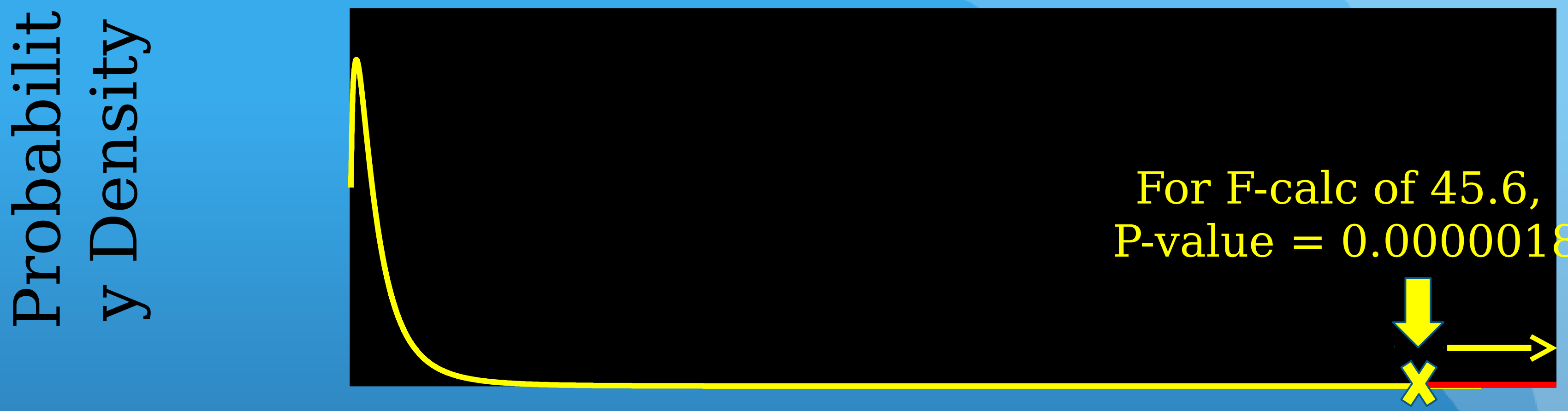
p-value

"F test": H_0 = All treatment means are equal



ANOVA table

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Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
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Milking practice	3	17.1	5.7	45.6	<0.001
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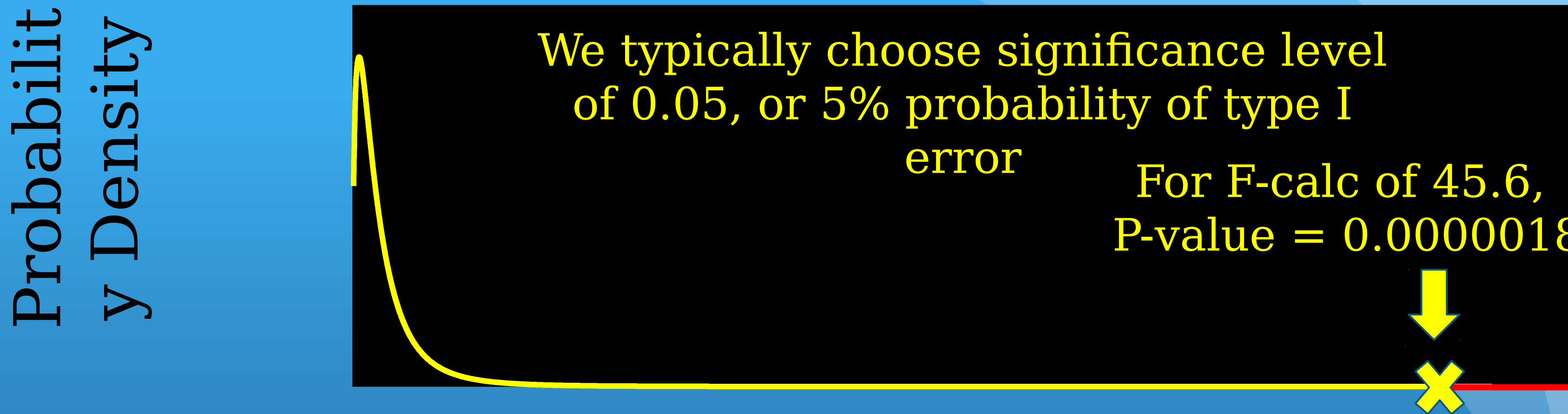
“F test”: H_0 = All treatment means are equal



ANOVA table

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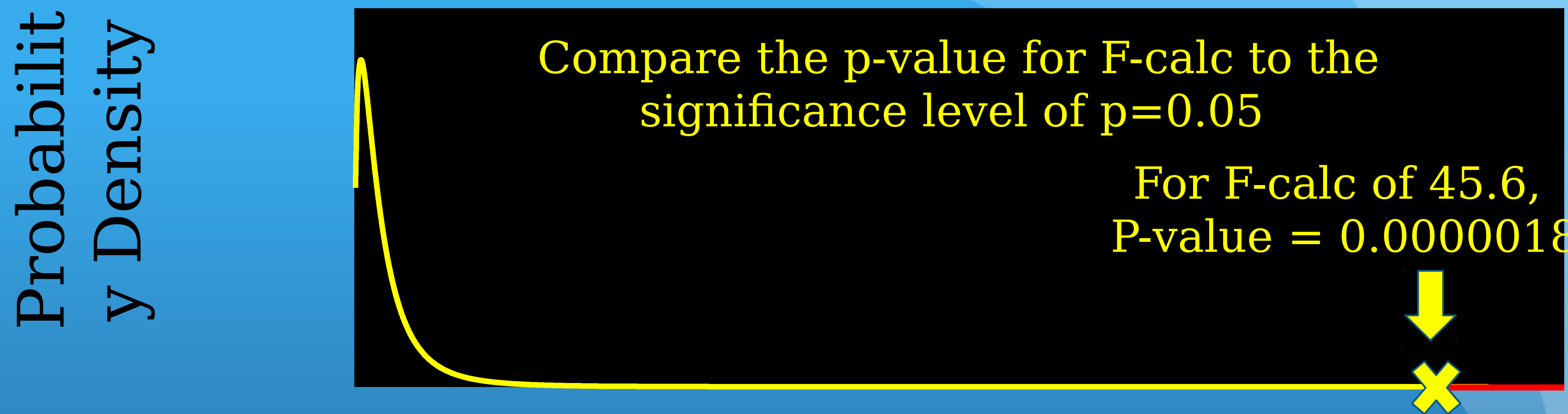
“F test”: H_0 = All treatment means are equal



ANOVA table

Response (Y_{ij}) = milk production

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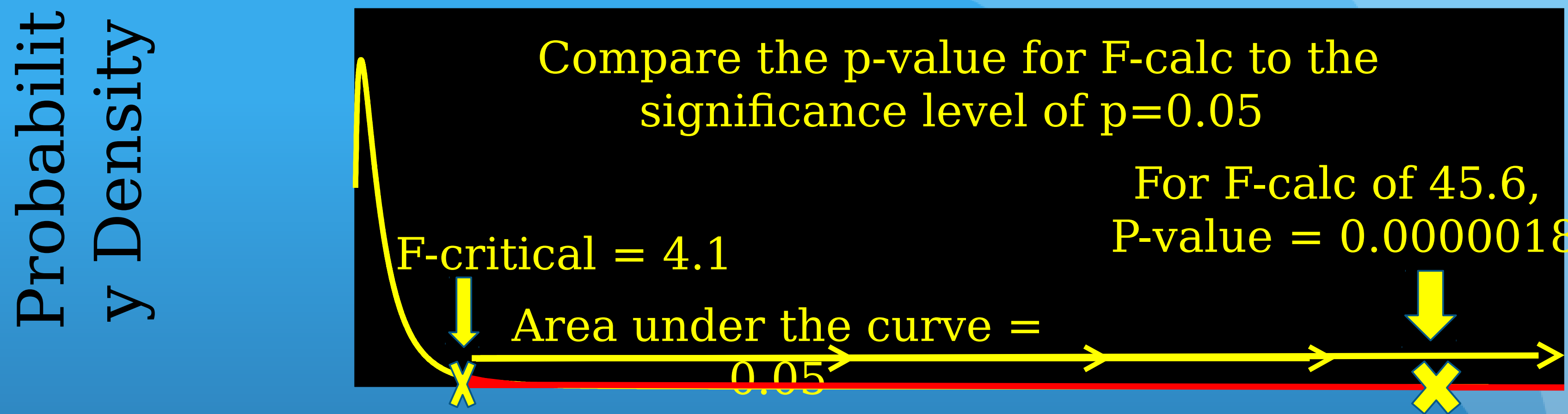
“F test”: H_0 = All treatment means are equal



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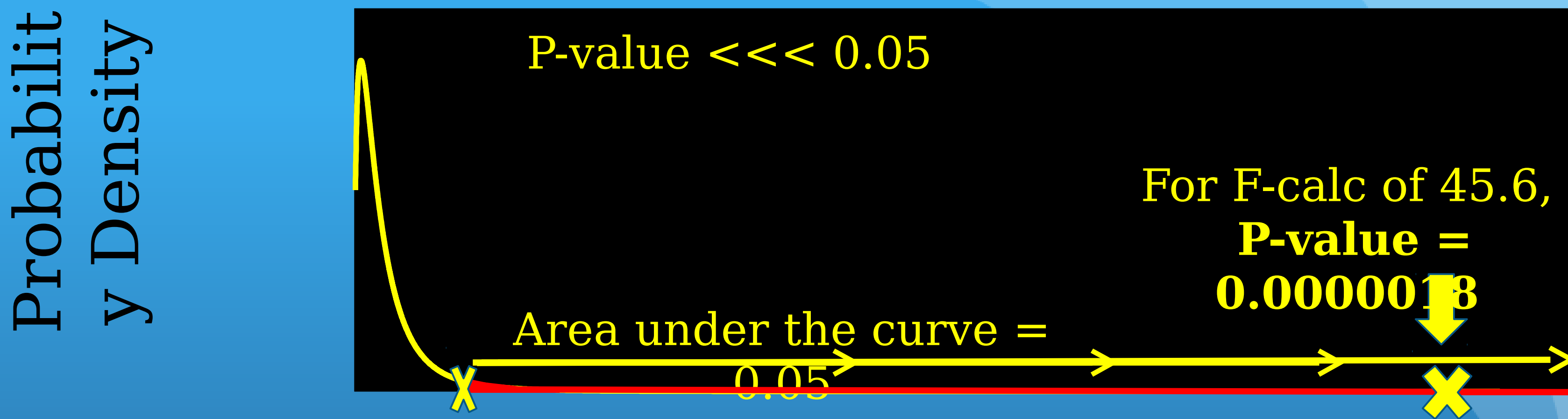
Error	8	1	0.125		

p-value

“F test”: H_0 = All treatment means are equal



ANOVA table

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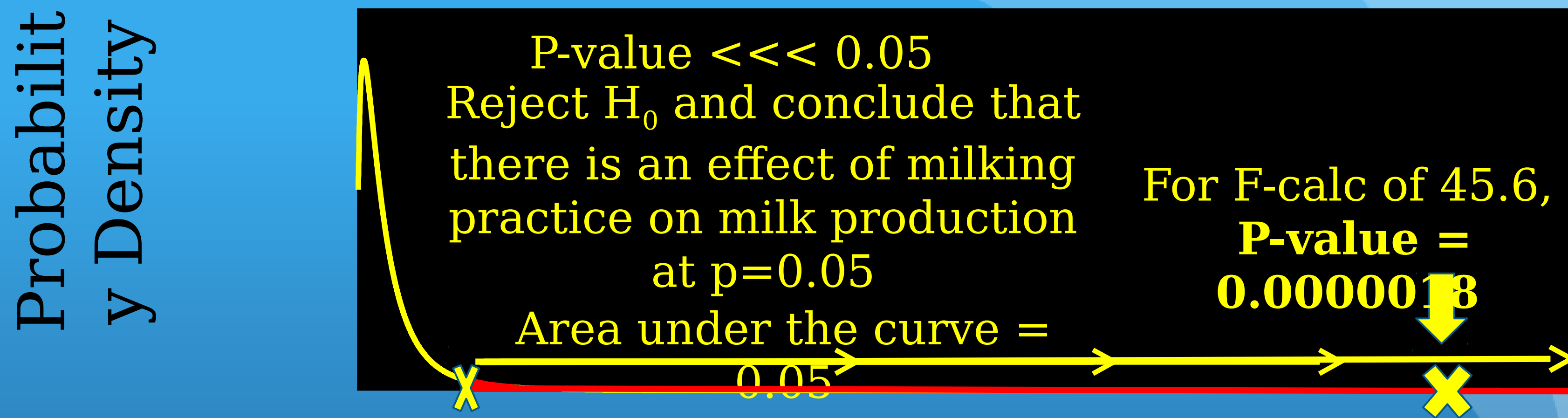
Milking practice	3	17.1	5.7	45.6	<0.001
Error	8	1	0.125		

p-value

"F test": ~~H_0 = All treatment means are equal~~



ANOVA table

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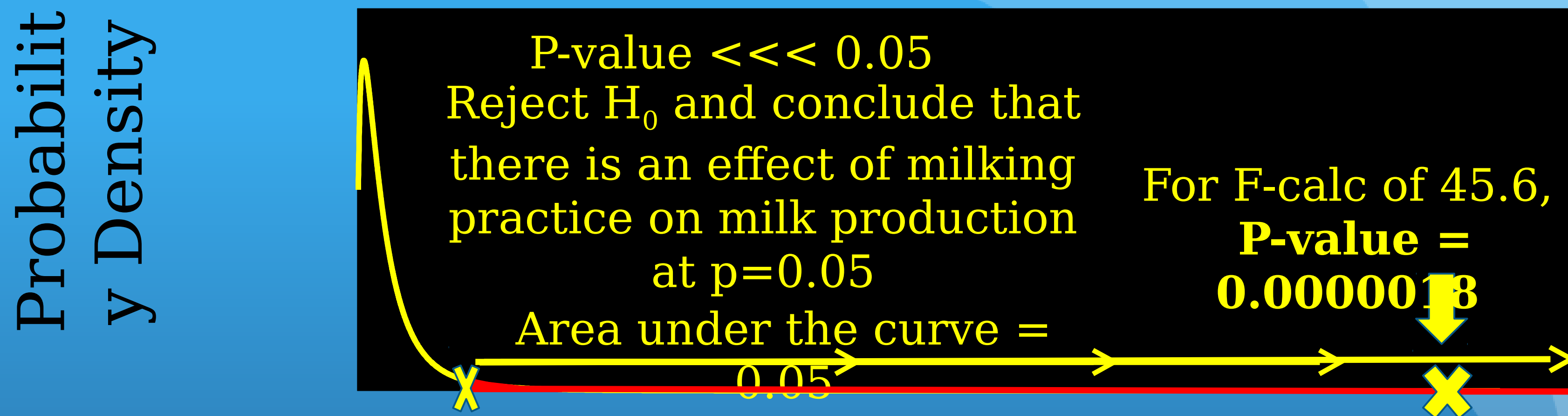
Error	8	1	0.125		

p-value

"F test": ~~H_0 = All treatment means are equal~~



ANOVA table

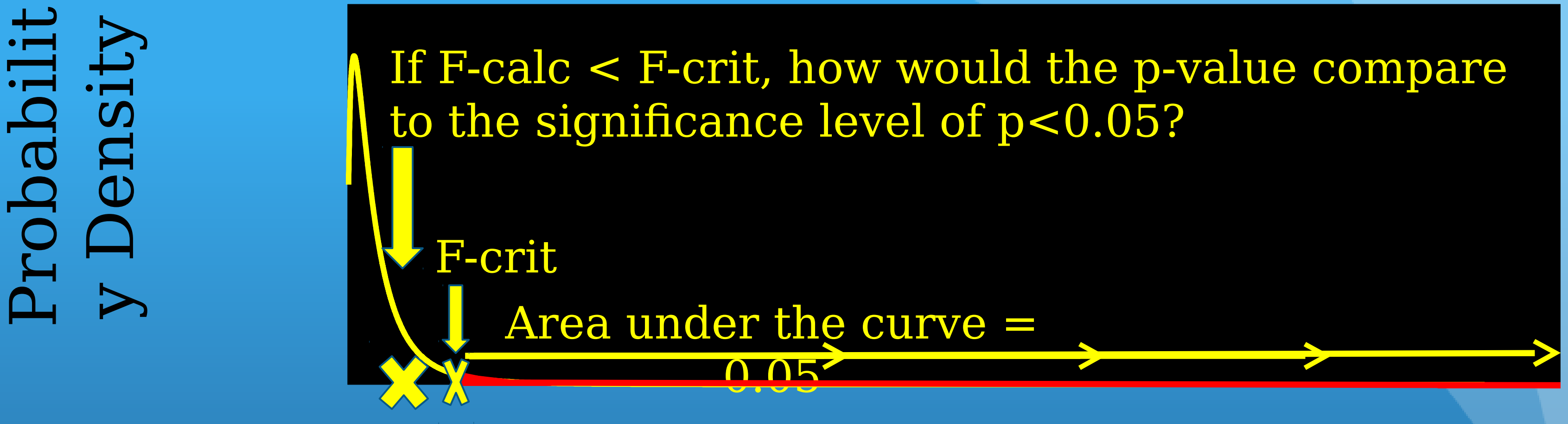
Response (Y_{ij}) = milk production

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Sq (MS)**	**F value**	**P value (Pr(>p))**
Total	11	18.1			
Milking practice	3	17.1	5.7	45.6	<0.001***
Error	8	1	0.125		

*** = highly significant

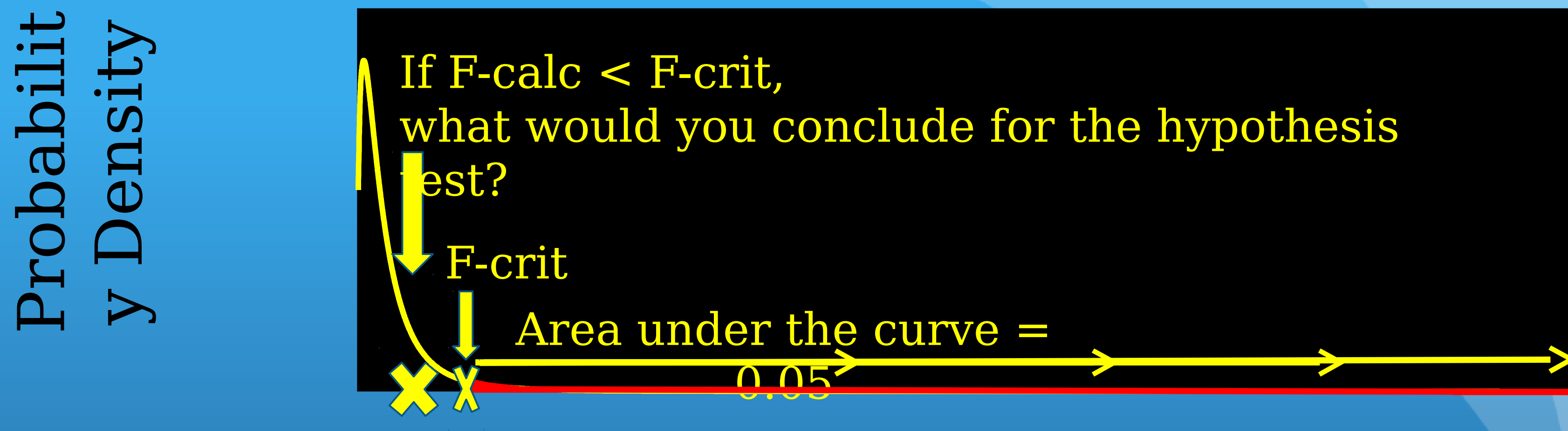
“F test”: H_0 = All treatment means are equal

If F-calc < F-crit, how would the p-value compare to the significance level of p<0.05?

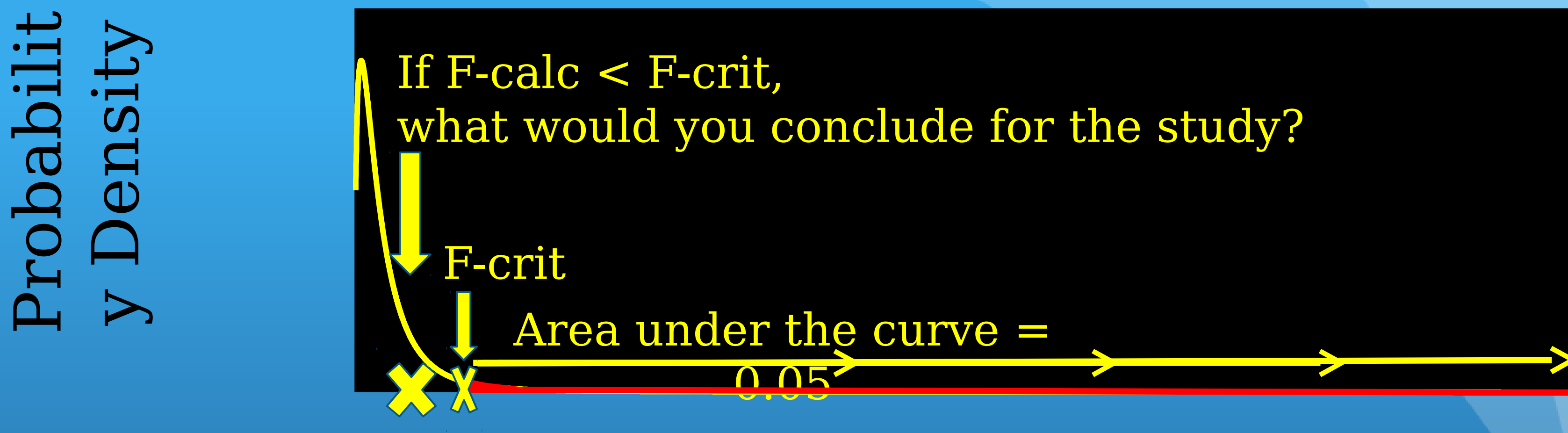


“F test”: H_0 = All treatment means are equal

If F-calc < F-crit,
what would you conclude for the hypothesis test?



"F test": H_0 = All treatment means are equal



Section 1 Learning Objectives

You should now have a basic understanding of:

1. Elements of an experiment
   - Experimental unit, independent variable, dependent variable, randomization
2. Complete Randomized Design (CRD)
3. Statistical model for a CRD with one treatment
4. Principles of Analysis of Variance (ANOVA) for a CRD with one treatment
   - Elements in an ANOVA table for a CRD with one treatment (Sources of variation, Sums of Squares, Mean Squares, F-value, P-value)
   - Hypothesis testing using F-test

We concluded that milking practice has an effect on milk production ($p<0.05$)

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

But *which* milking practices differ?

	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

We will learn how to do all of this using the AIP App after the break!

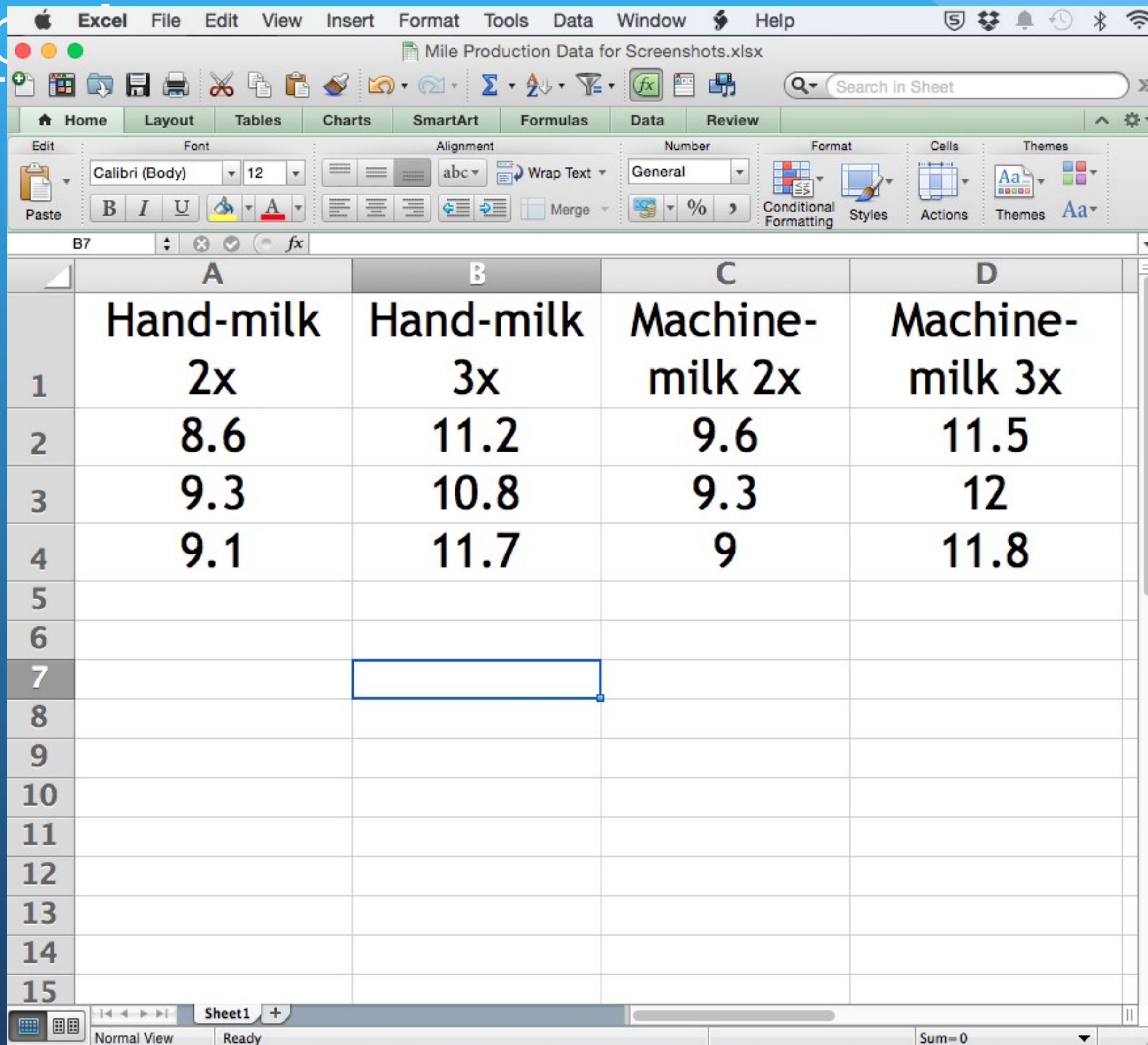
	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
	8.6 	11.2 	9.6 	11.5 
	9.3 	10.8 	9.3 	12.0 
	9.1 	11.7 	9.0 	11.8 
Treatment means:	9.0	11.2	9.3	11.8

Grand mean = 9.3

Section 2 Learning Objectives

1. Know how to use the AIP App to analyze a CRD
   - Formatting and loading CSV file
   - Testing assumptions (later), ANOVA, LSD mean separation
2. Know how to interpret results

How to format data file for AIP inp



First note, column versus row.

B7	A	B	C	D
1	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
2	8.6	11.2	9.6	11.5
3	9.3	10.8	9.3	12
4	9.1	11.7	9	11.8
5				
6				
7				
8				
9				

Four columns

	A	B	C	D
1	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
2	8.6	11.2	9.6	11.5
3	9.3	10.8	9.3	12
4	9.1	11.7	9	11.8
5				
6				
7				
8				
9				

Four rows

B7

	A	B	C	D
1	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
2	8.6	11.2	9.6	11.5
3	9.3	10.8	9.3	12
4	9.1	11.7	9	11.8
5				
6				
7				
8				
9				

Header row shows the titles of each column

B7 *fx*

	A	B	C	D
1	Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
2	8.6	11.2	9.6	11.5
3	9.3	10.8	9.3	12
4	9.1	11.7	9	11.8
5				
6				
7				
8				
9				

Formatting data file for App:
Dependent data (response variable) must be cut and paste to a single column and independent data (treatments) in a separate column.

Hand-milk 2x	Hand-milk 3x	Machine-milk 2x	Machine-milk 3x
8.6	11.2	9.6	11.5
9.3	10.8	9.3	12
9.1	11.7	9	11.8

Independent variable = Treatment, "milking practice"

Dependent variable = Milk production

Independe nt variable = Treatment, “milking practice”

Dependent variable = Milk production

	A	B	C	D
1	Treatment	Milk production		
2	Hand-milk 2x	8.6		
3	Hand-milk 2x	9.3		
4	Hand-milk 2x	9.1		
5	Hand-milk 3x	11.2		
6	Hand-milk 3x	10.8		
7	Hand-milk 3x	11.7		
8	Machine-milk 2x	9.6		
9	Machine-milk 2x	9.3		
10	Machine-milk 2x	9		
11	Machine-milk 3x	11.5		
12	Machine-milk 3x	12		
13	Machine-milk 3x	11.8		
14				

Vertical (Value) Axis

Milk production data.csv

Ready

Sum=0

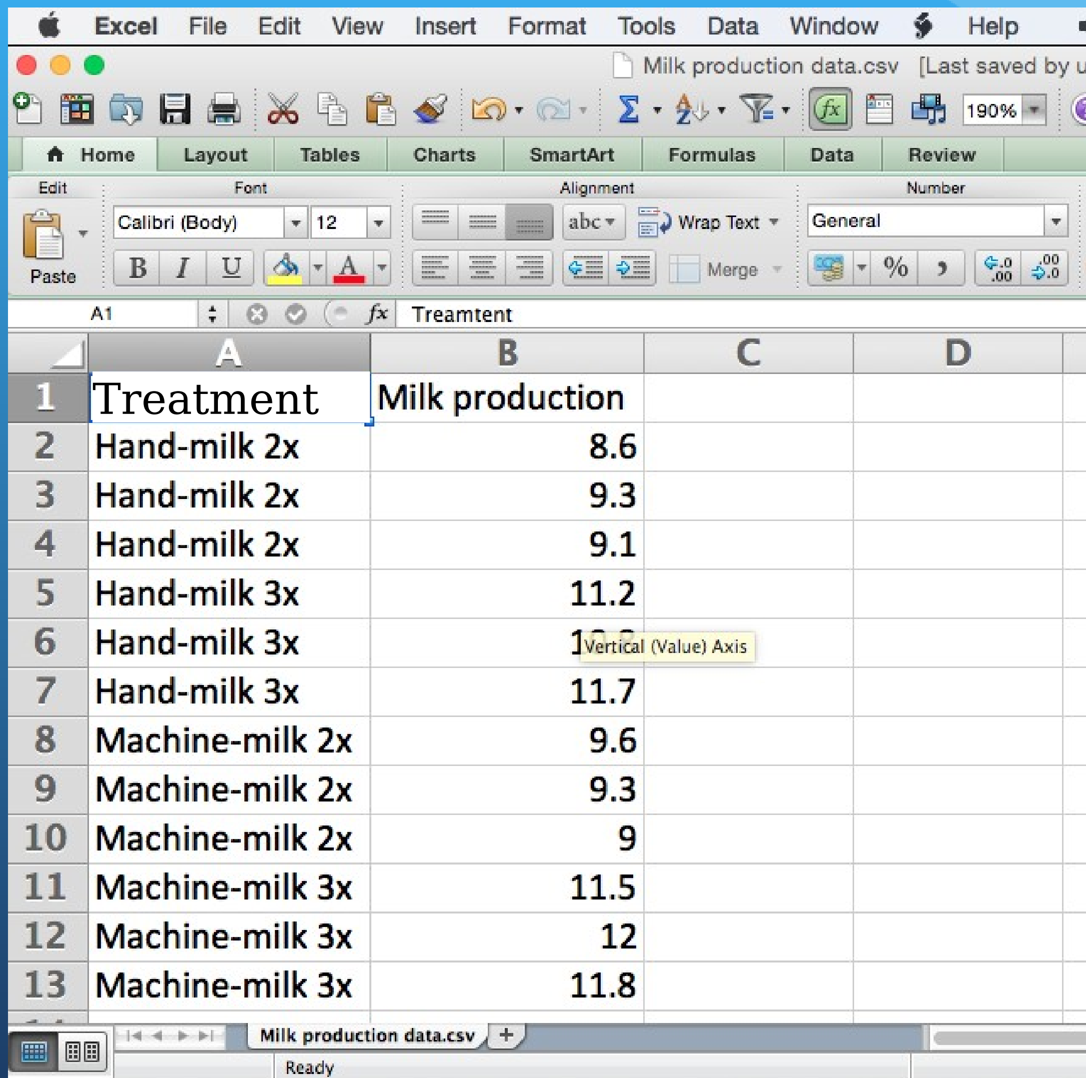
includes the (1) treatment (independent variable) and the (2) observation (dependent variable). If there were other independent variables (e.g. block, more treatments), they would have there own columns to identify all information for each record.

	A	B	C	D
1	Treatment	Milk production		
2	Hand-milk 2x	8.6		
3	Hand-milk 2x	9.3		
4	Hand-milk 2x	9.1		
5	Hand-milk 3x	11.2		
6	Hand-milk 3x	10.8		
7	Hand-milk 3x	11.7		
8	Machine-milk 2x	9.6		
9	Machine-milk 2x	9.3		
10	Machine-milk 2x	9		
11	Machine-milk 3x	11.5		
12	Machine-milk 3x	12		
13	Machine-milk 3x	11.8		
14				

Milk production data.csv

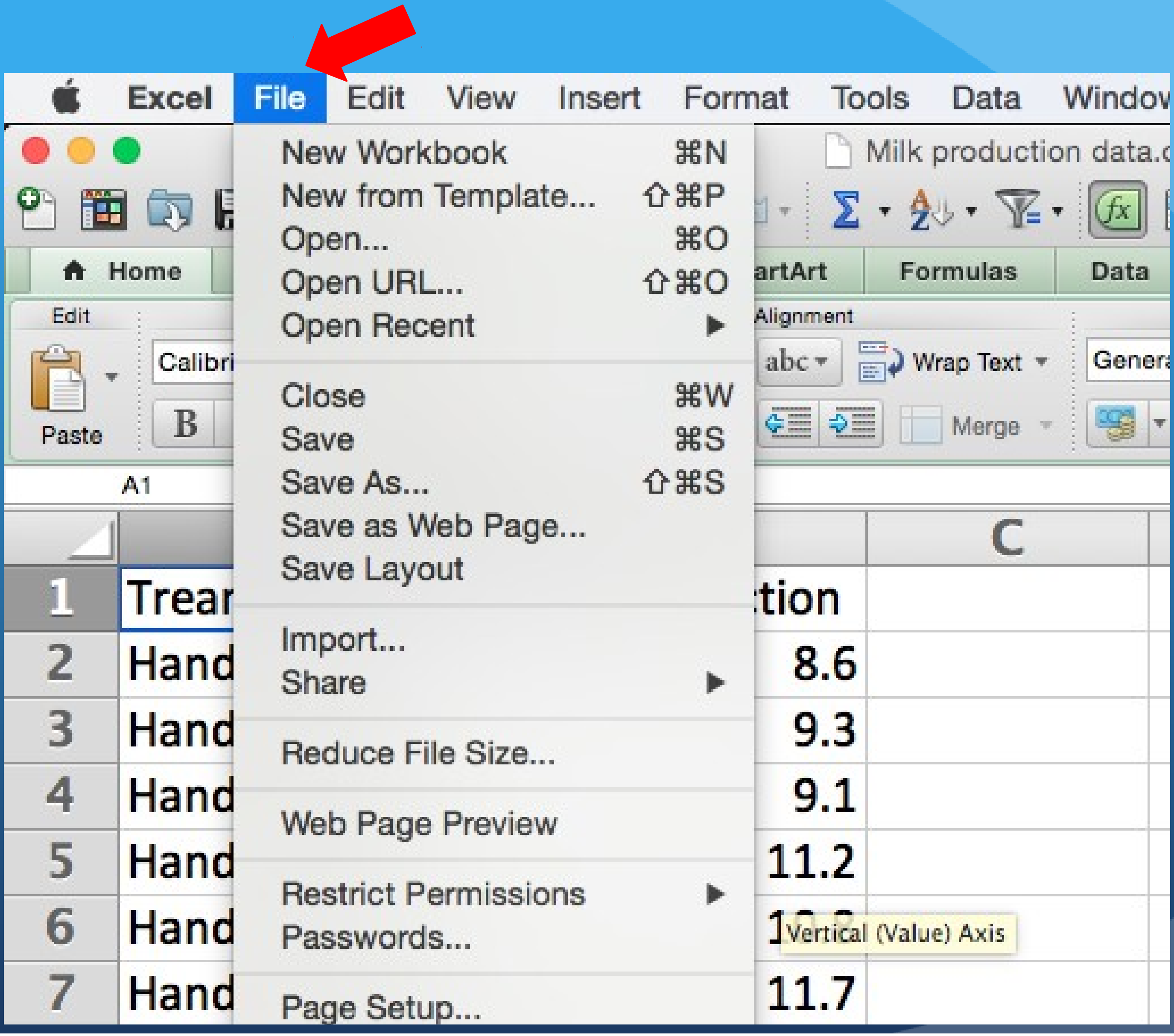
Record for each observation

How to save as .csv file?

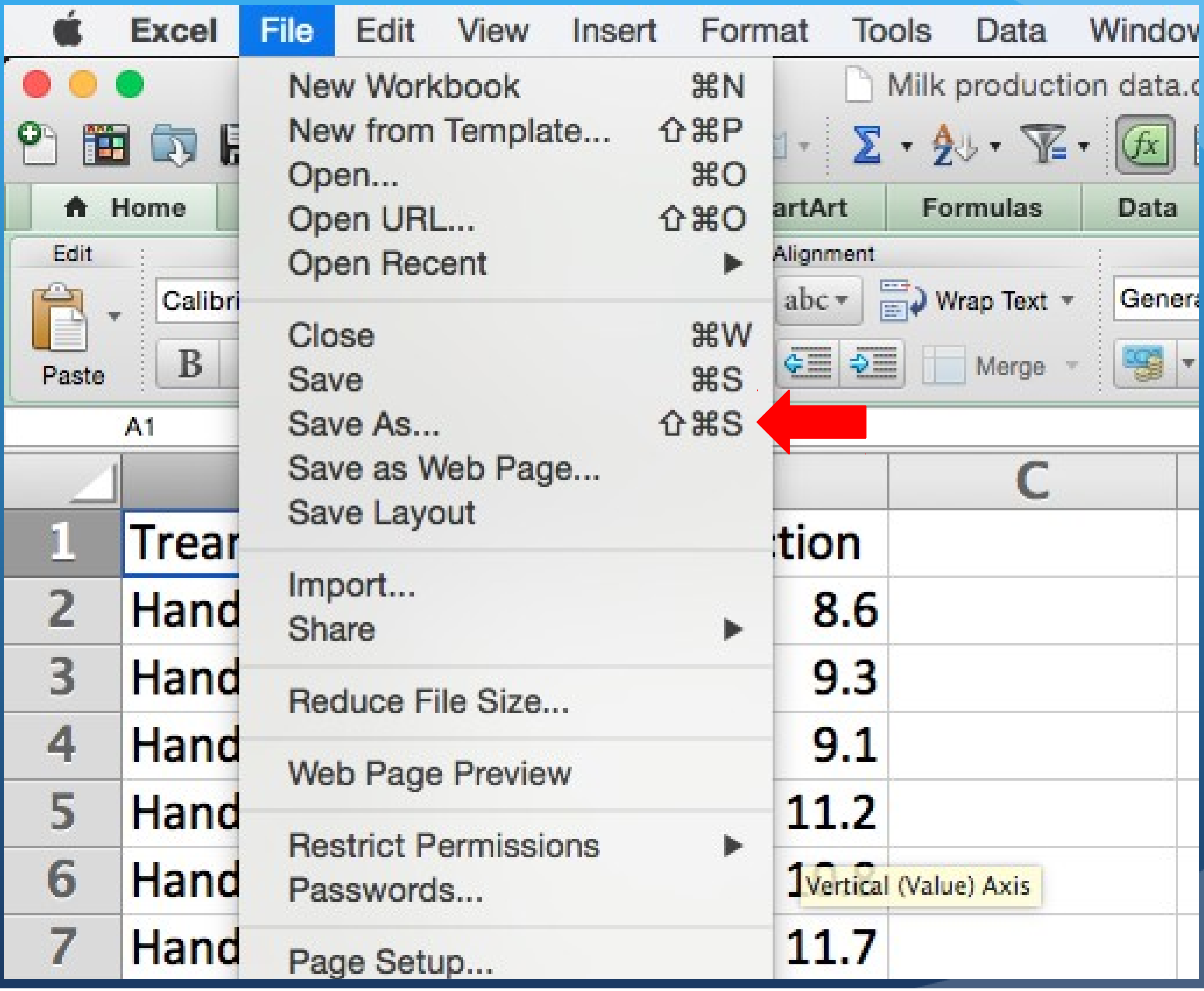


	A	B
1	Treatment	Milk production
2	Hand-milk 2x	8.6
3	Hand-milk 2x	9.3
4	Hand-milk 2x	9.1
5	Hand-milk 3x	11.2
6	Hand-milk 3x	[illegible]
7	Hand-milk 3x	11.7
8	Machine-milk 2x	9.6
9	Machine-milk 2x	9.3
10	Machine-milk 2x	9
11	Machine-milk 3x	11.5
12	Machine-milk 3x	12
13	Machine-milk 3x	11.8

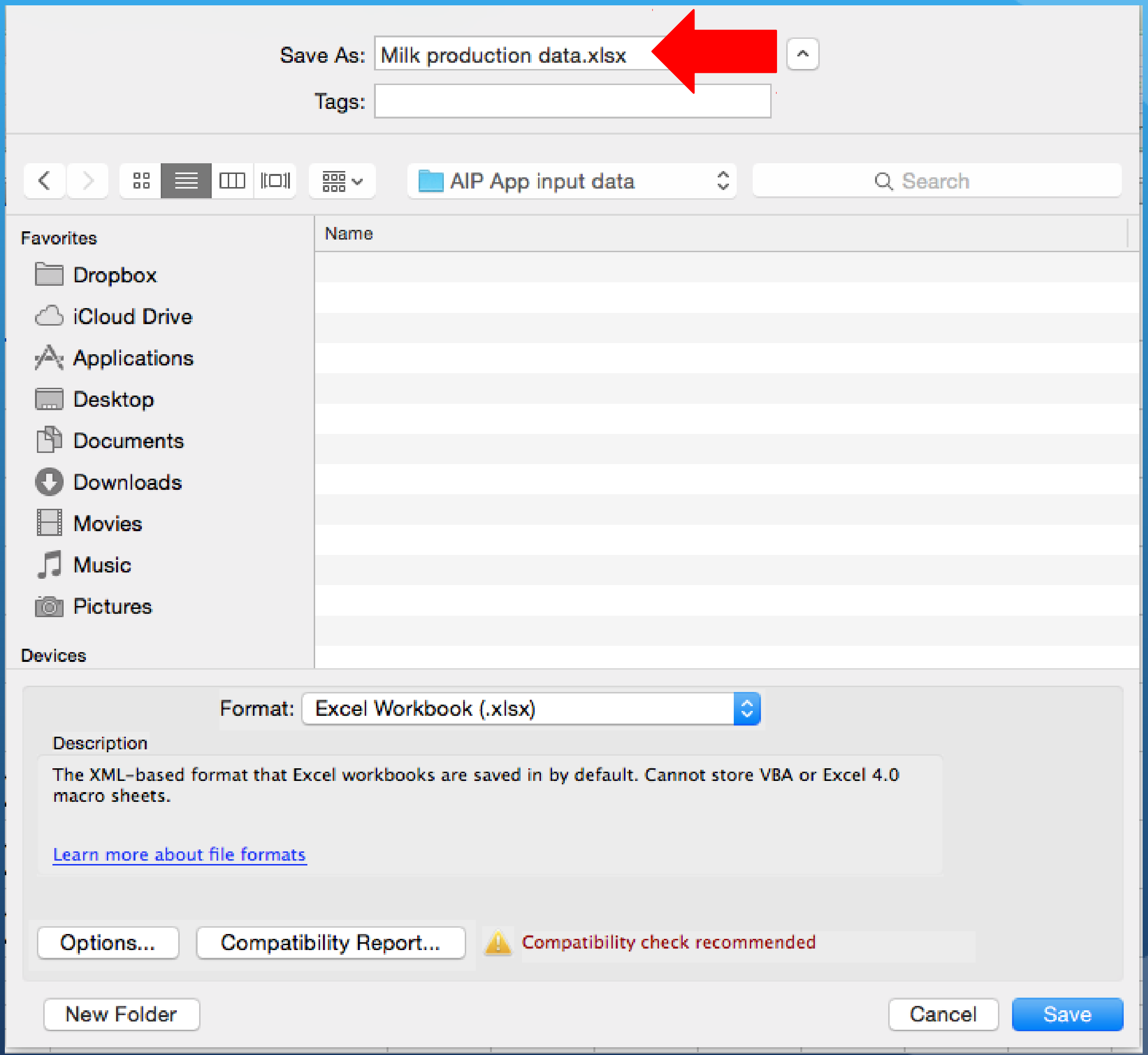
1. Click File


Excel
File
Edit
View
Insert
Format
Tools
Data
New Workbook ⌘N
New from Template... ⇧⌘P
Open... ⌘O
Open URL... ⇧⌘O
Open Recent
Close ⌘W
Save ⌘S
Save As... ⇧⌘S
Save as Web Page...
Save Layout
Import...
Share
Reduce File Size...
Web Page Preview
Restrict Permissions
Passwords...
Page Setup...
Milk production data.
Home
Formulas
Data
Edit
Paste
Calibri
Alignment
Wrap Text
Merge
A1
C
8.6
9.3
9.1
11.2
11.7
Vertical (Value) Axis

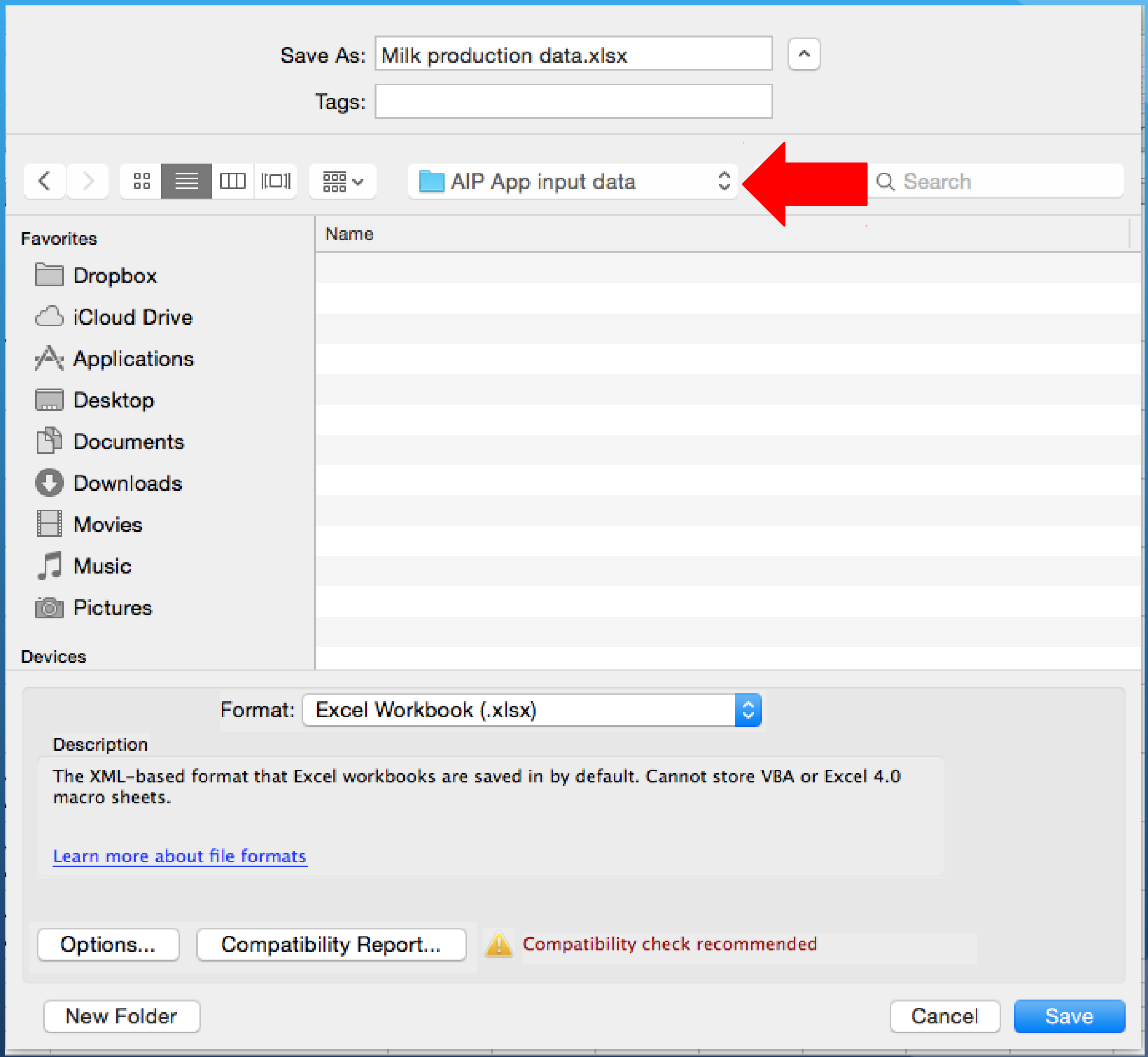
2. Click Save As…



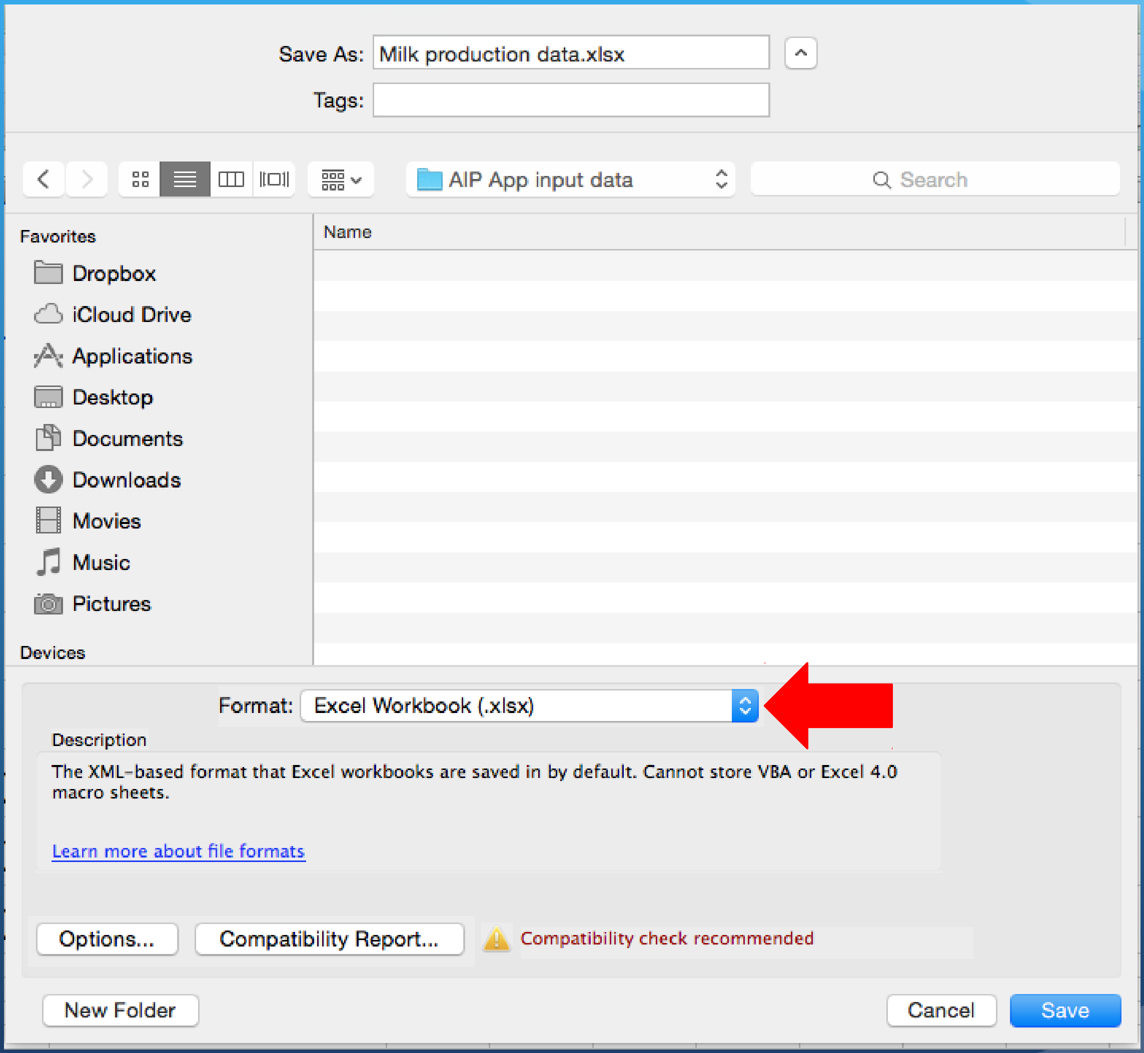
3. Enter filename



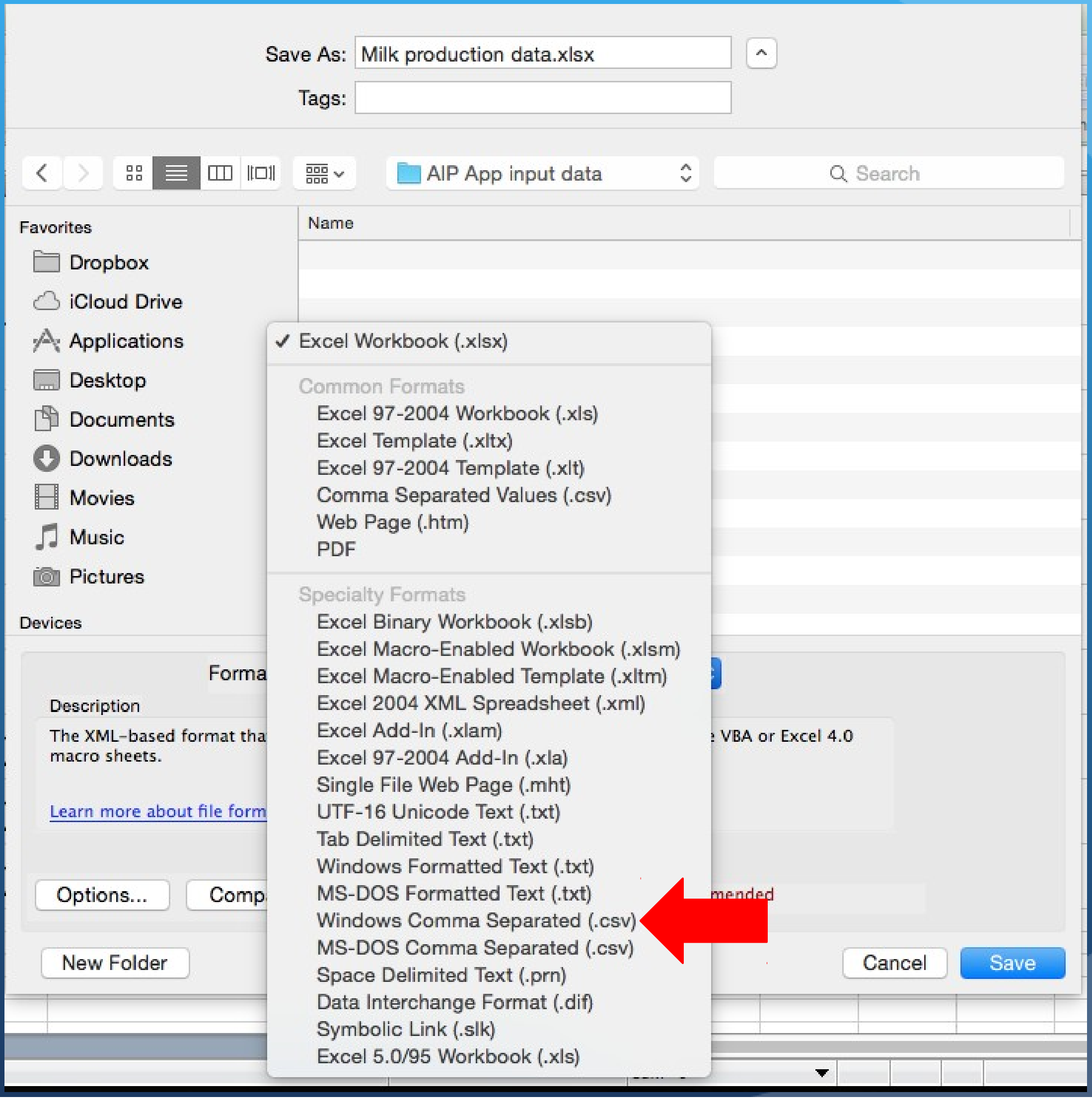
4. Note *where* you are saving the file. You will need to find it when you use the AIP app.



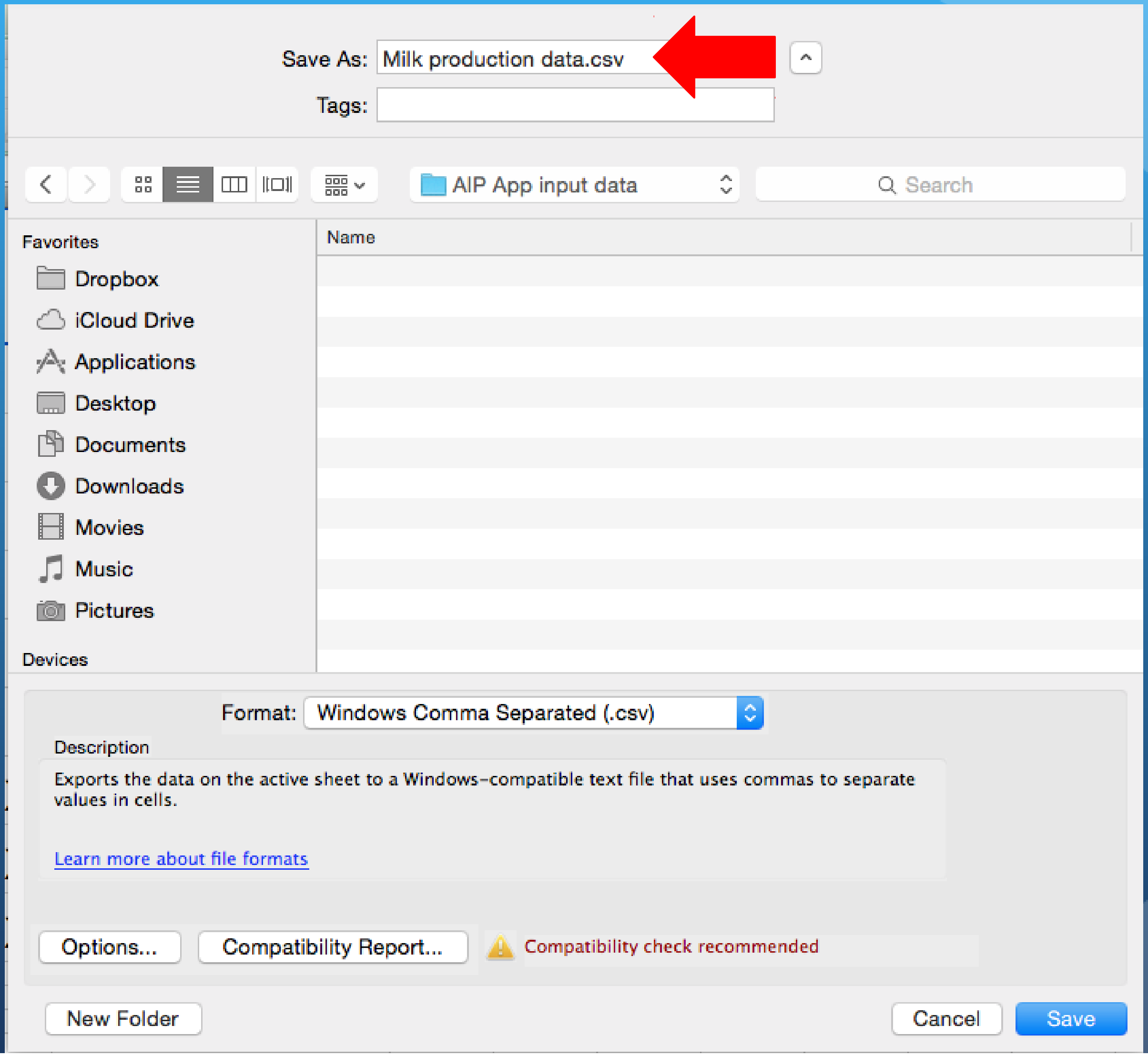
5. Click Format dropdown to select file type



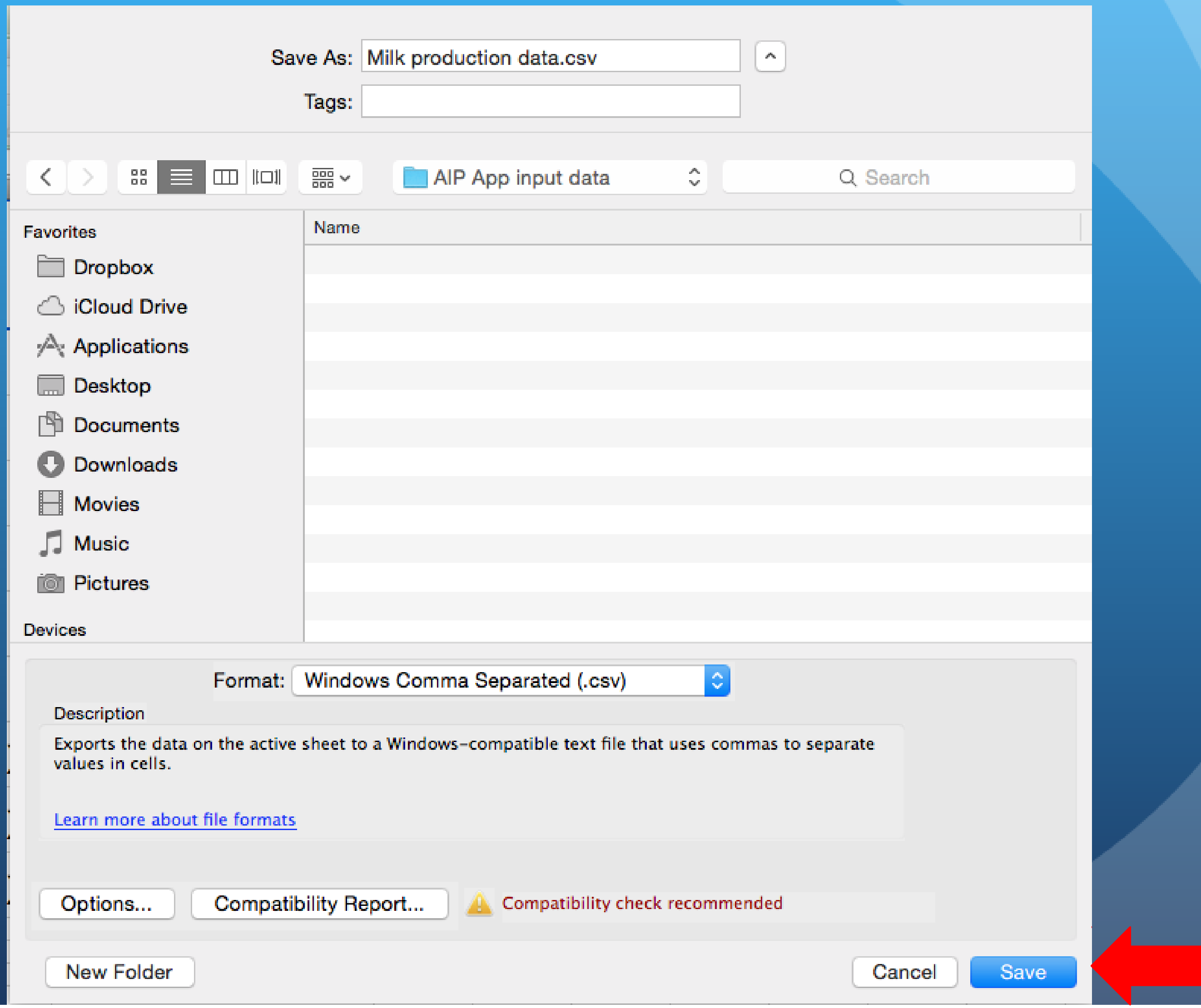
6. Click Windows Comma Separated (.csv)



7. Verify that your filename reads .csv



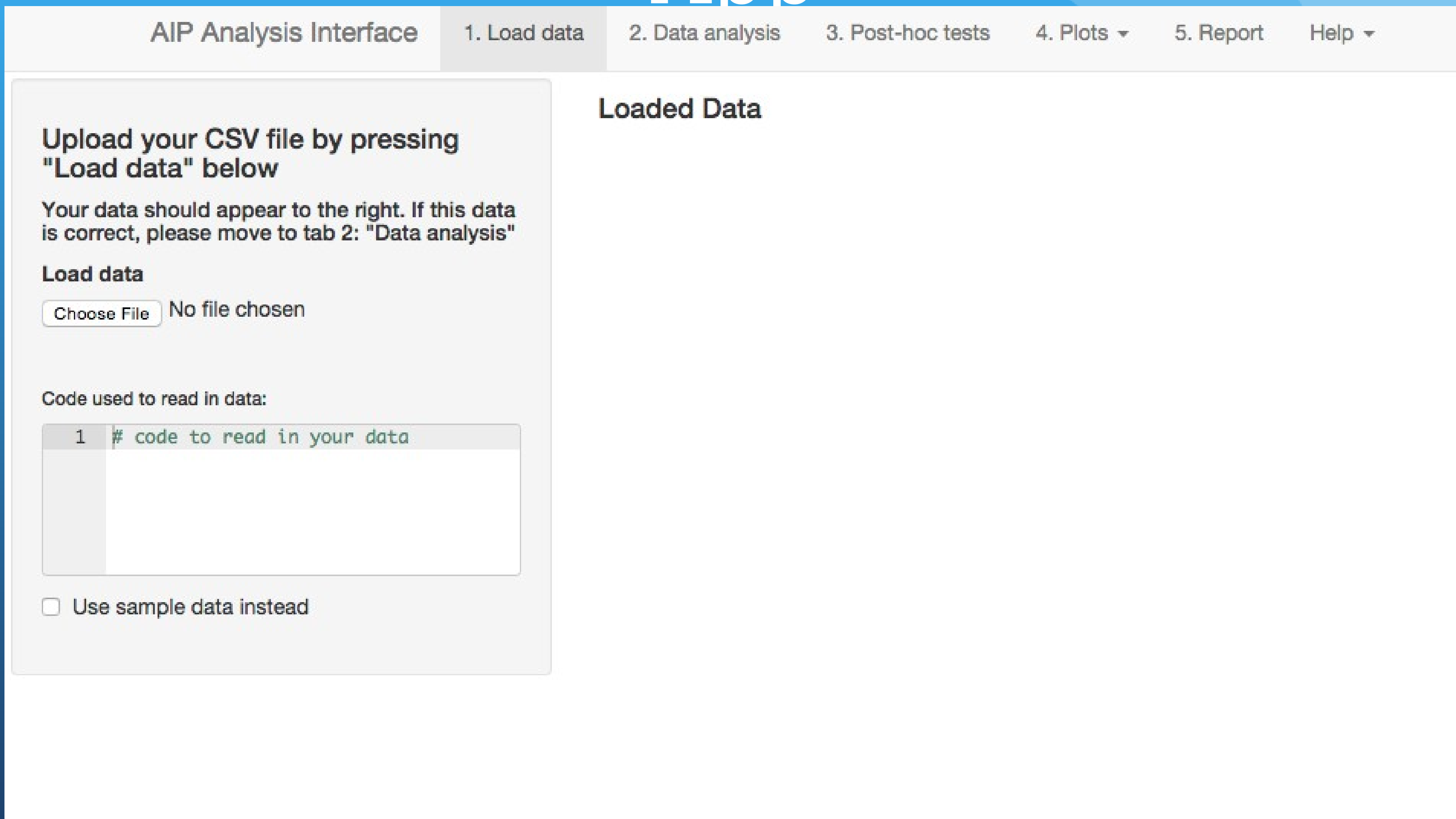
9. Lastly, click Save



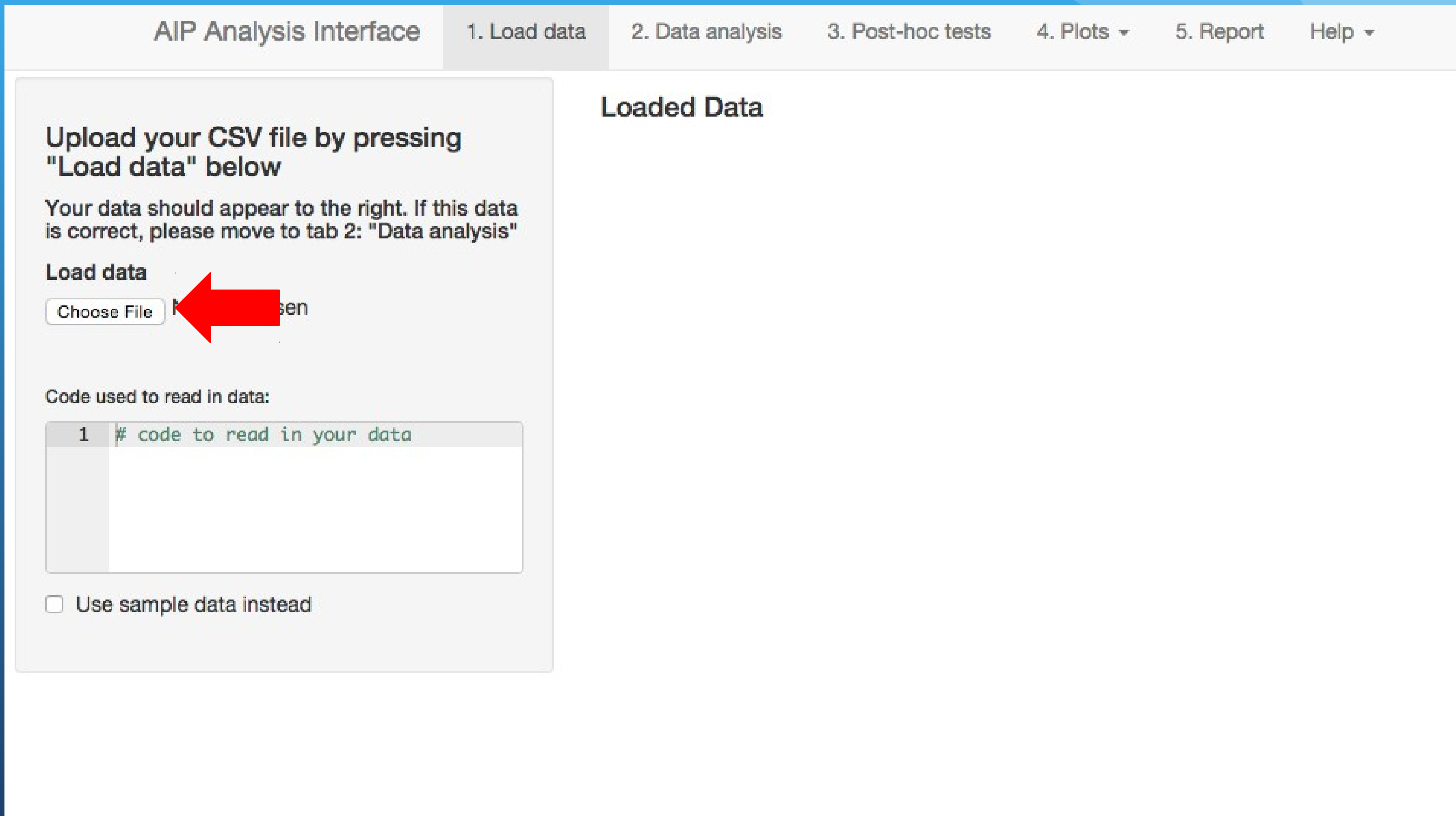
AIP App

- Simple and free
- We will learn how to:
  - How to format data in CSV file
  - Upload data to AIP app
  - Identify variables from CSV file and choose statistical model
  - Test assumptions of ANOVA
  - Post hoc tests
  - Plot results
  - Download statistics report and graphs

How to load data into AIP App



1. Click Choose File and select your csv file



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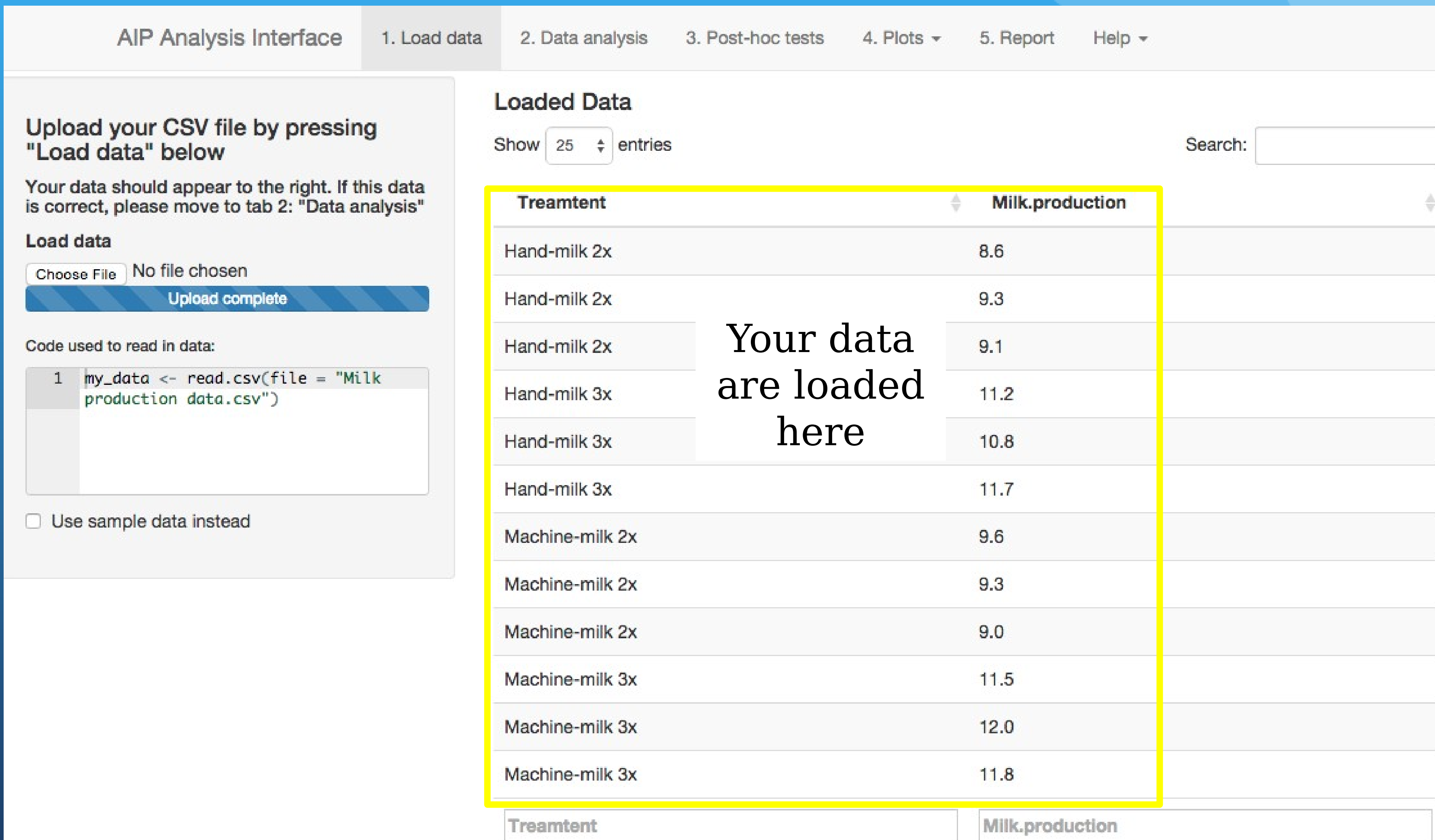
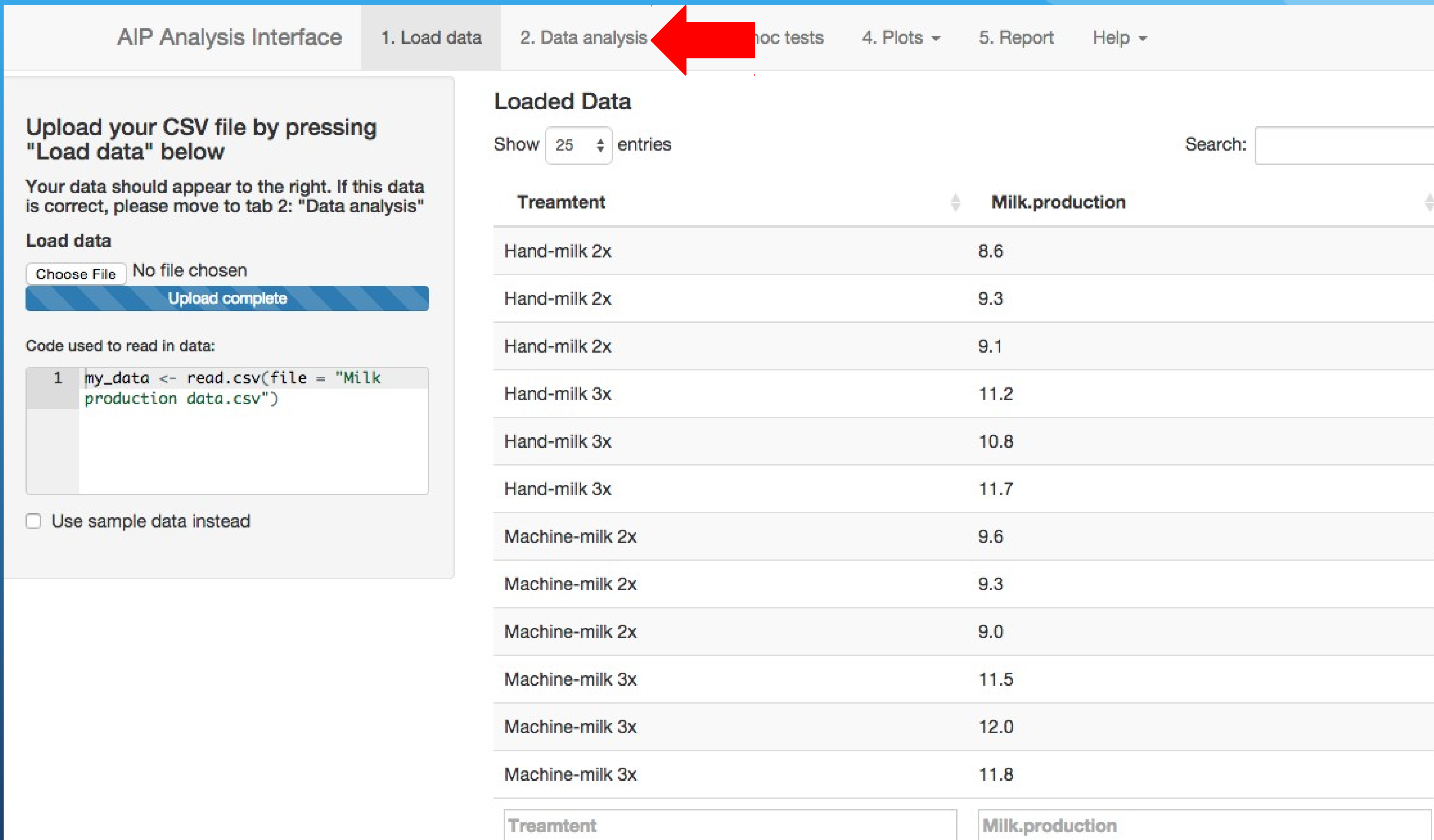
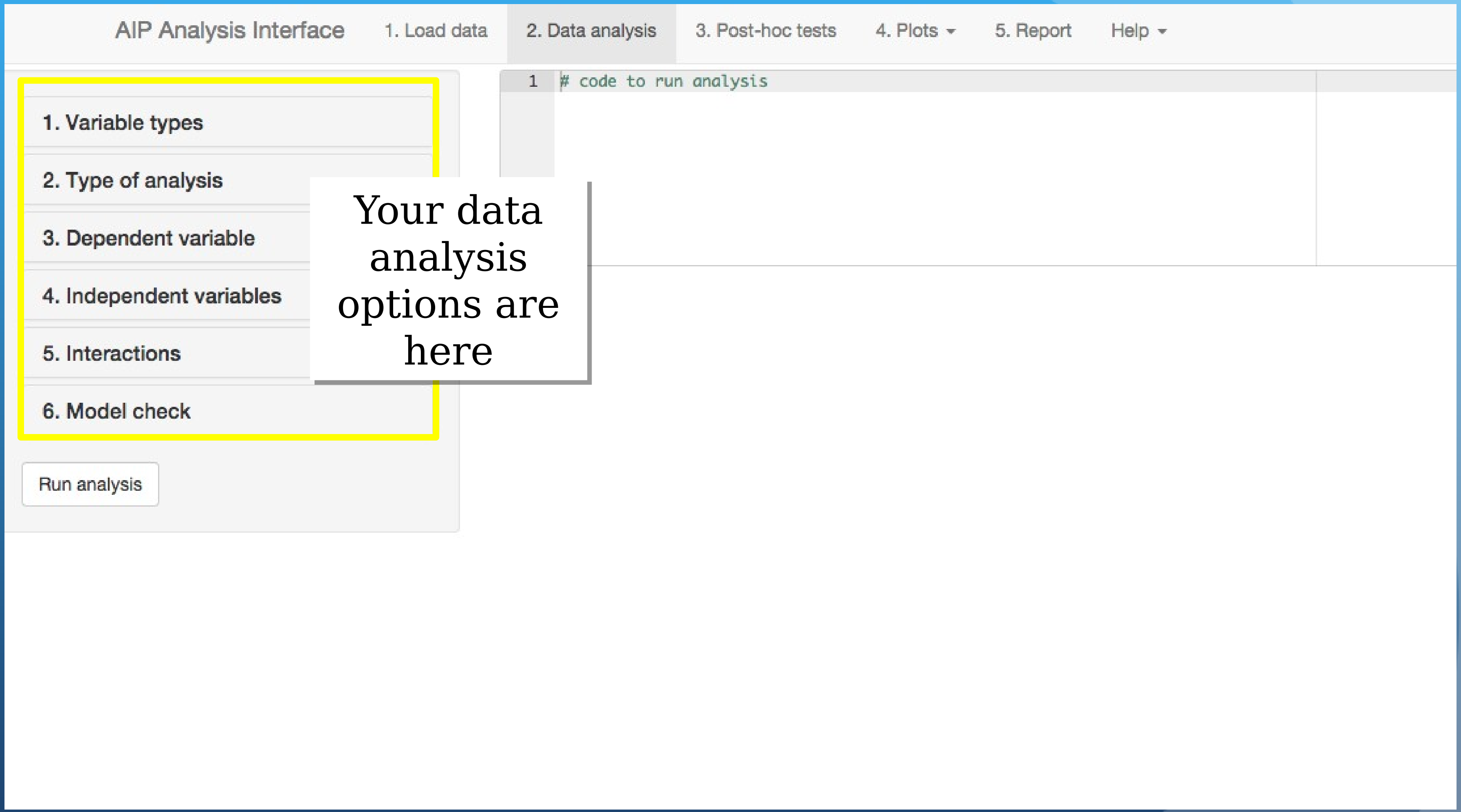


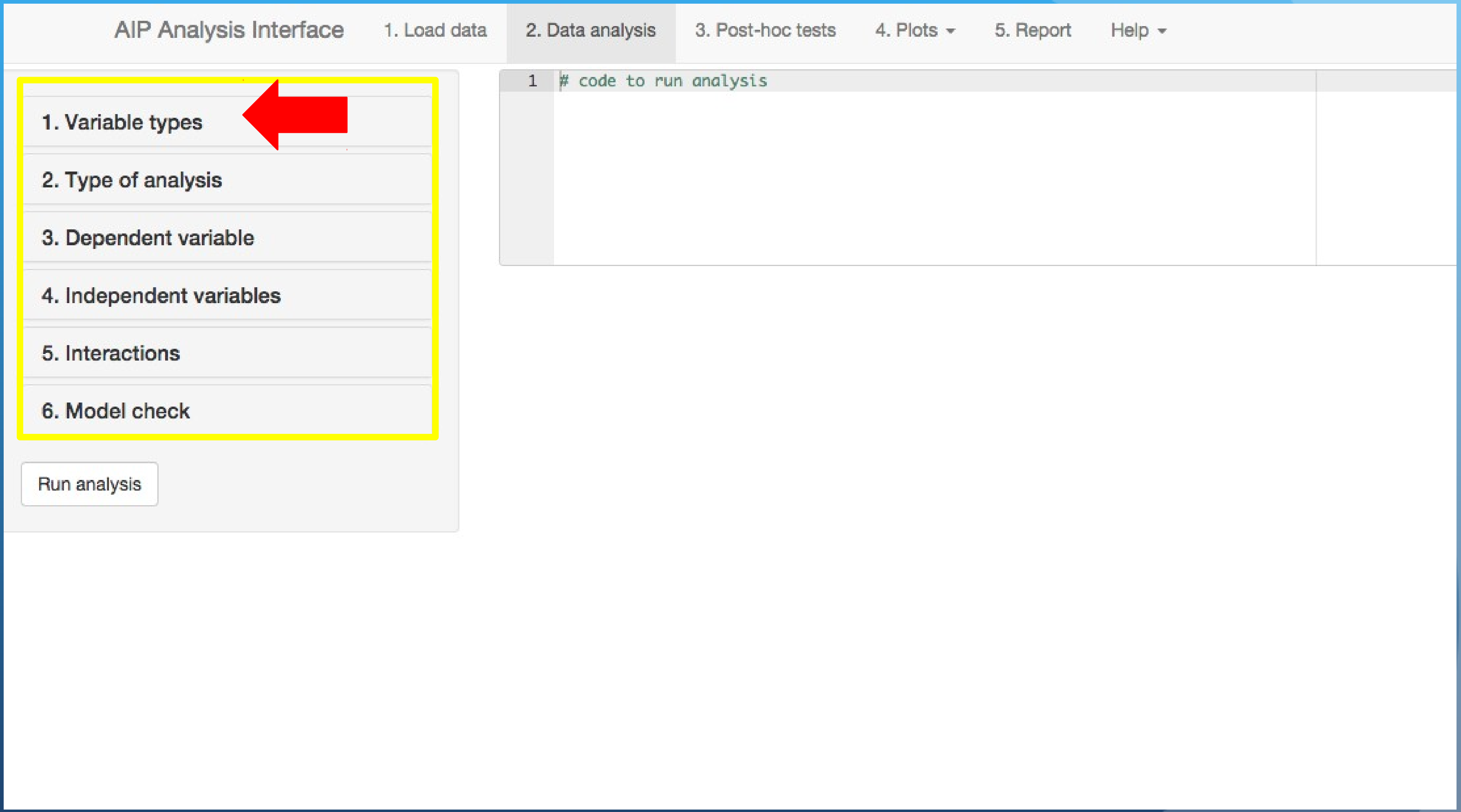
2. Click Data Analysis



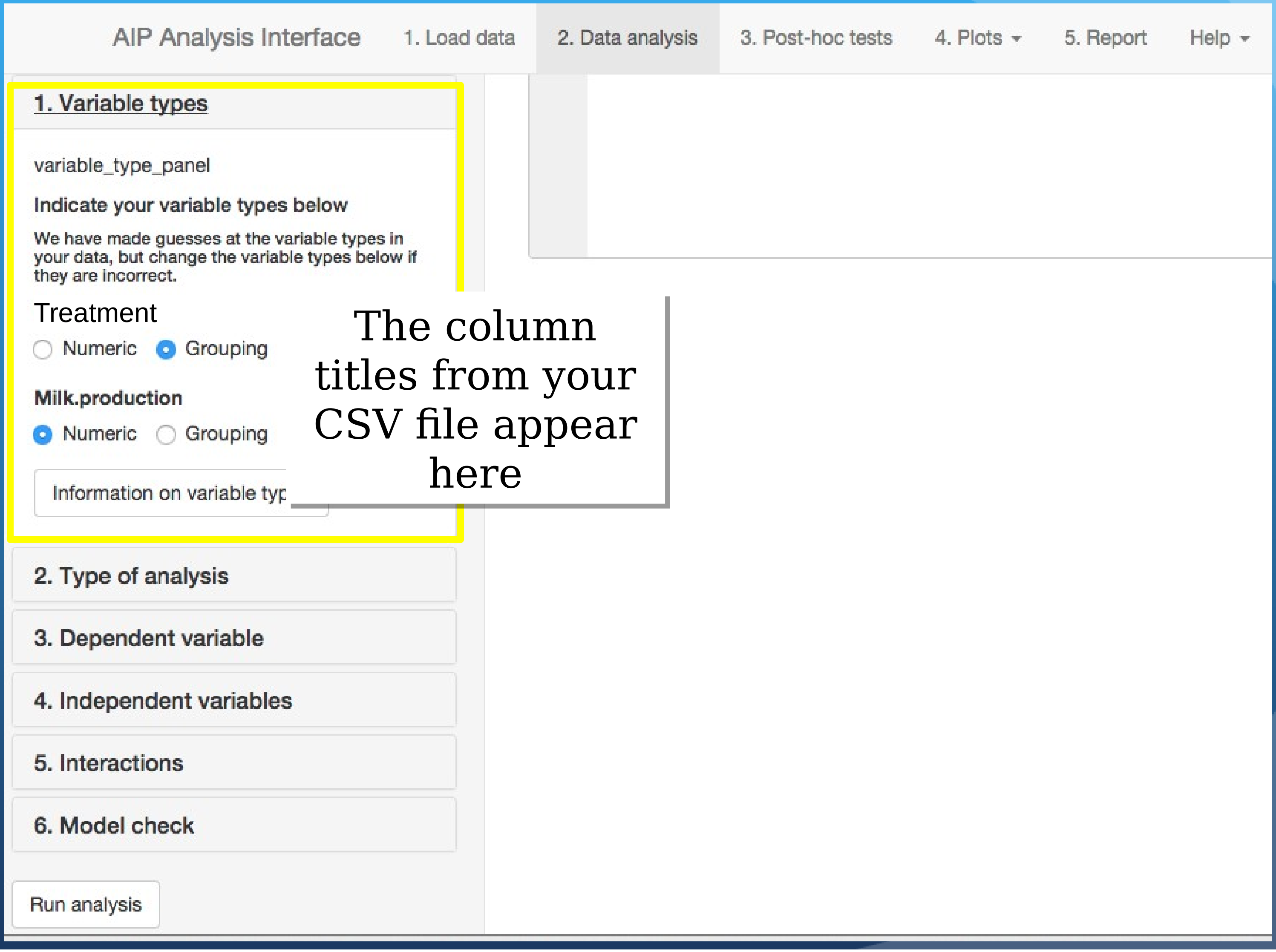
2. Click Data Analysis



3. Click Variable types

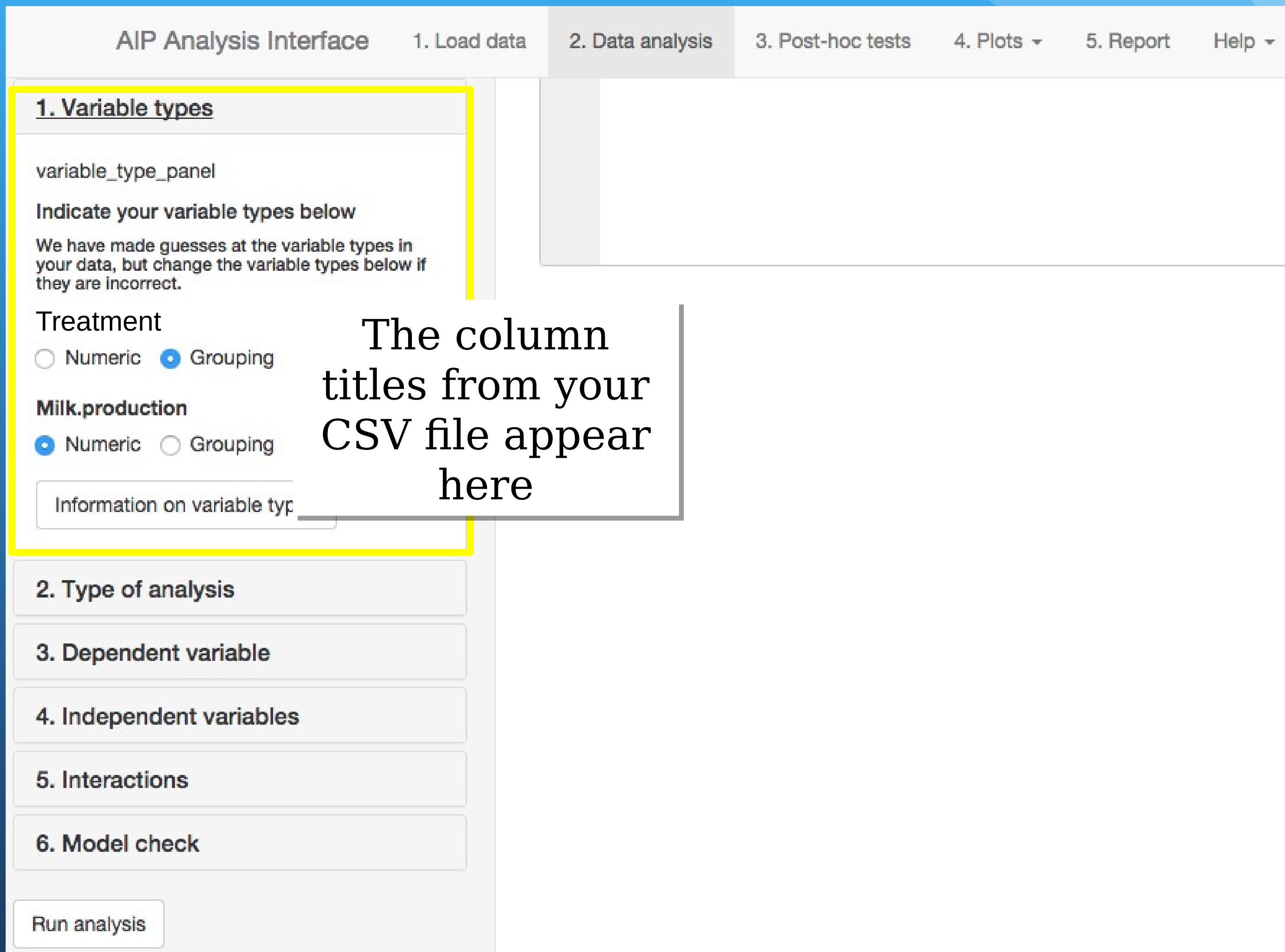


3. Click Variable types



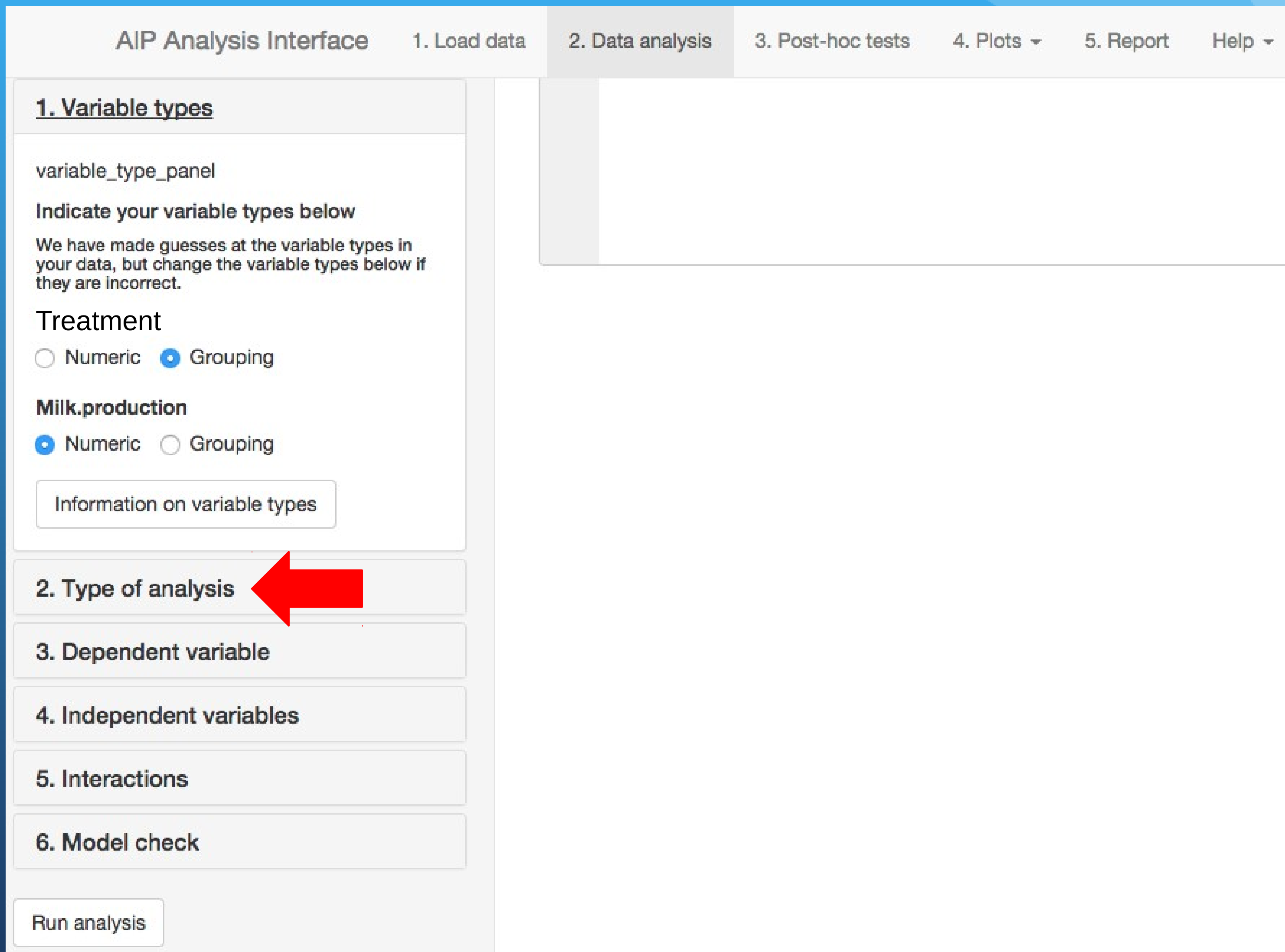
The column titles from your CSV file appear here

4. Choose the type of variable for each data column

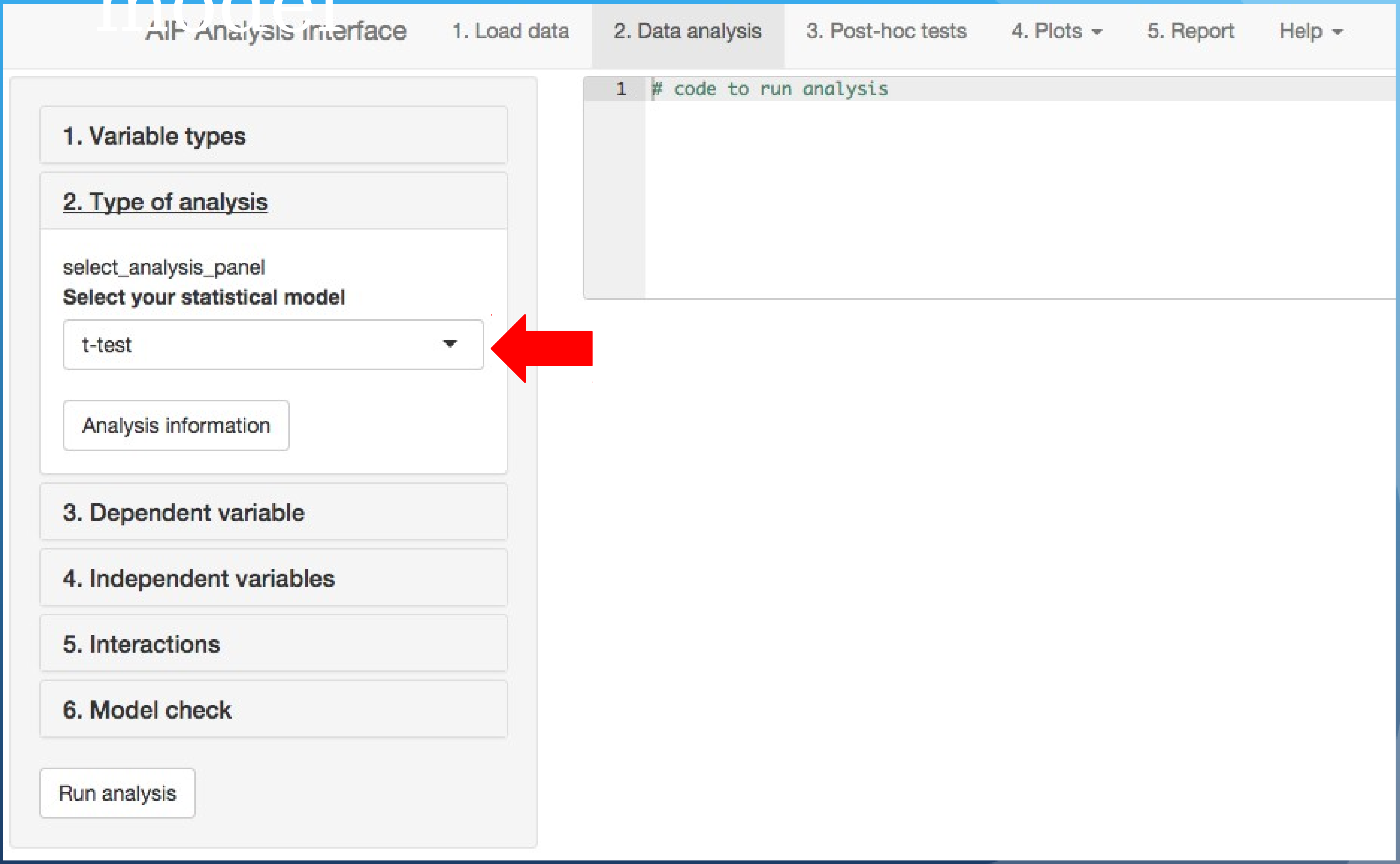


The column titles from your CSV file appear here

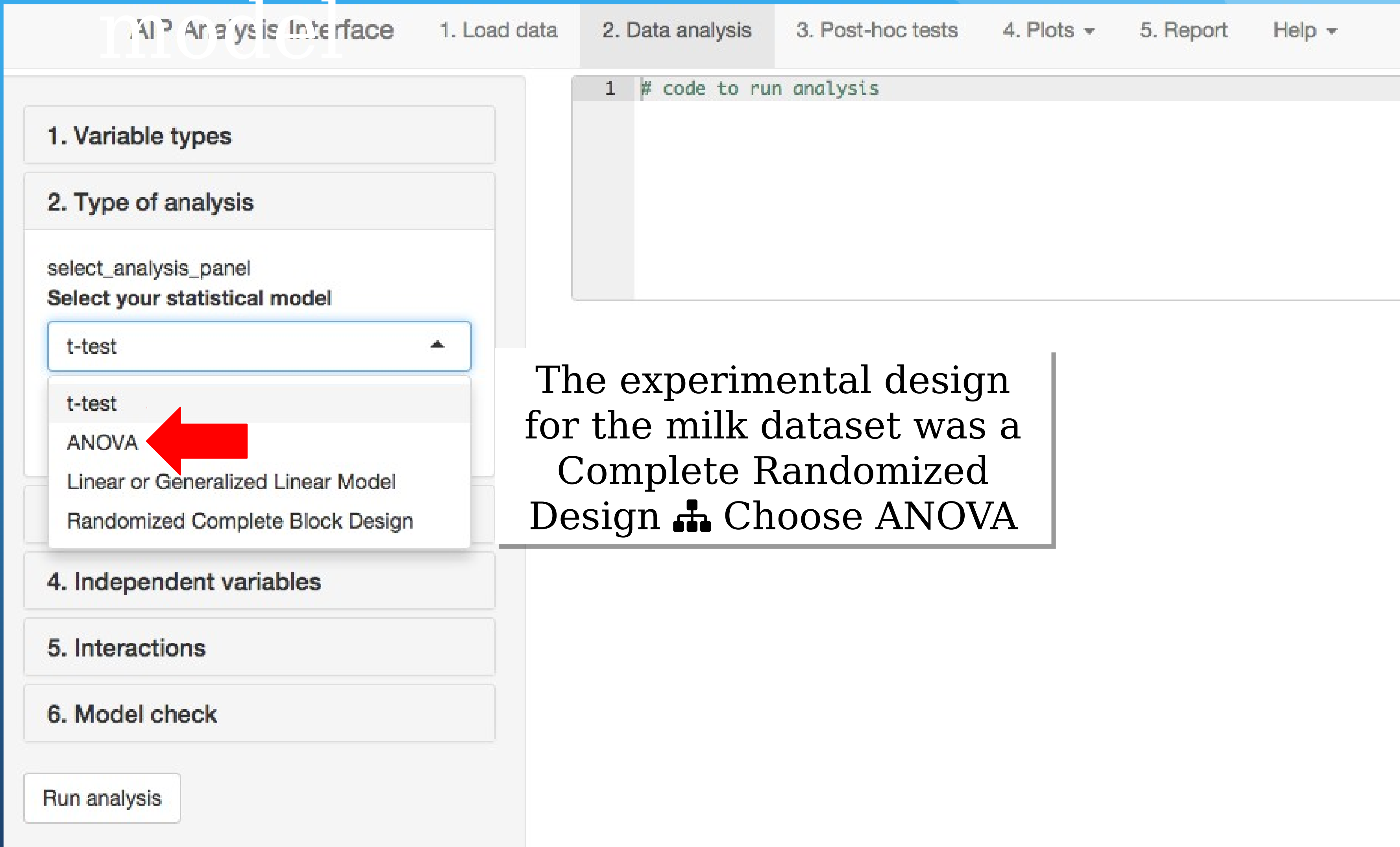
5. Click Type of Analysis



6. Select your statistical model

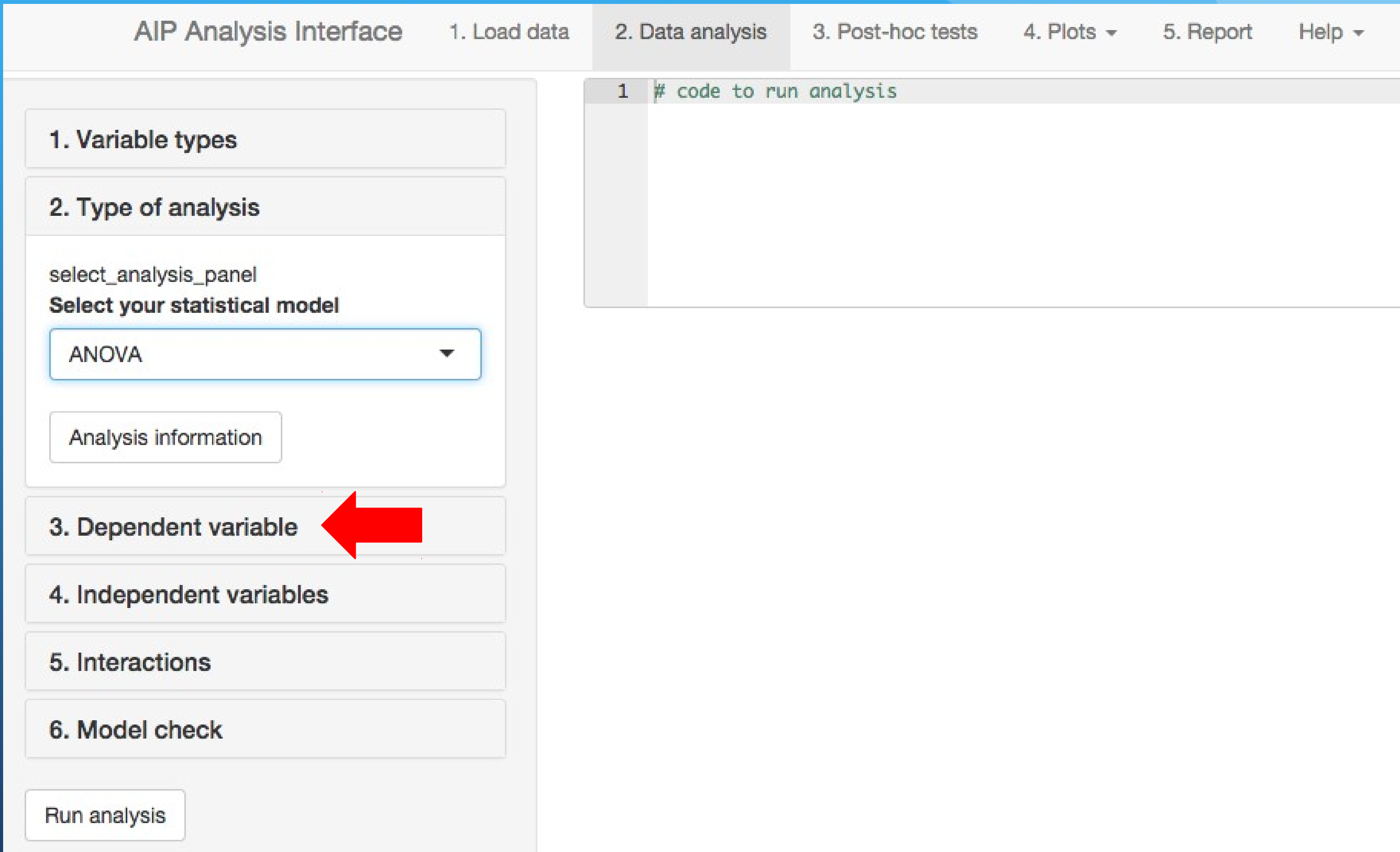


6. Select your statistical model

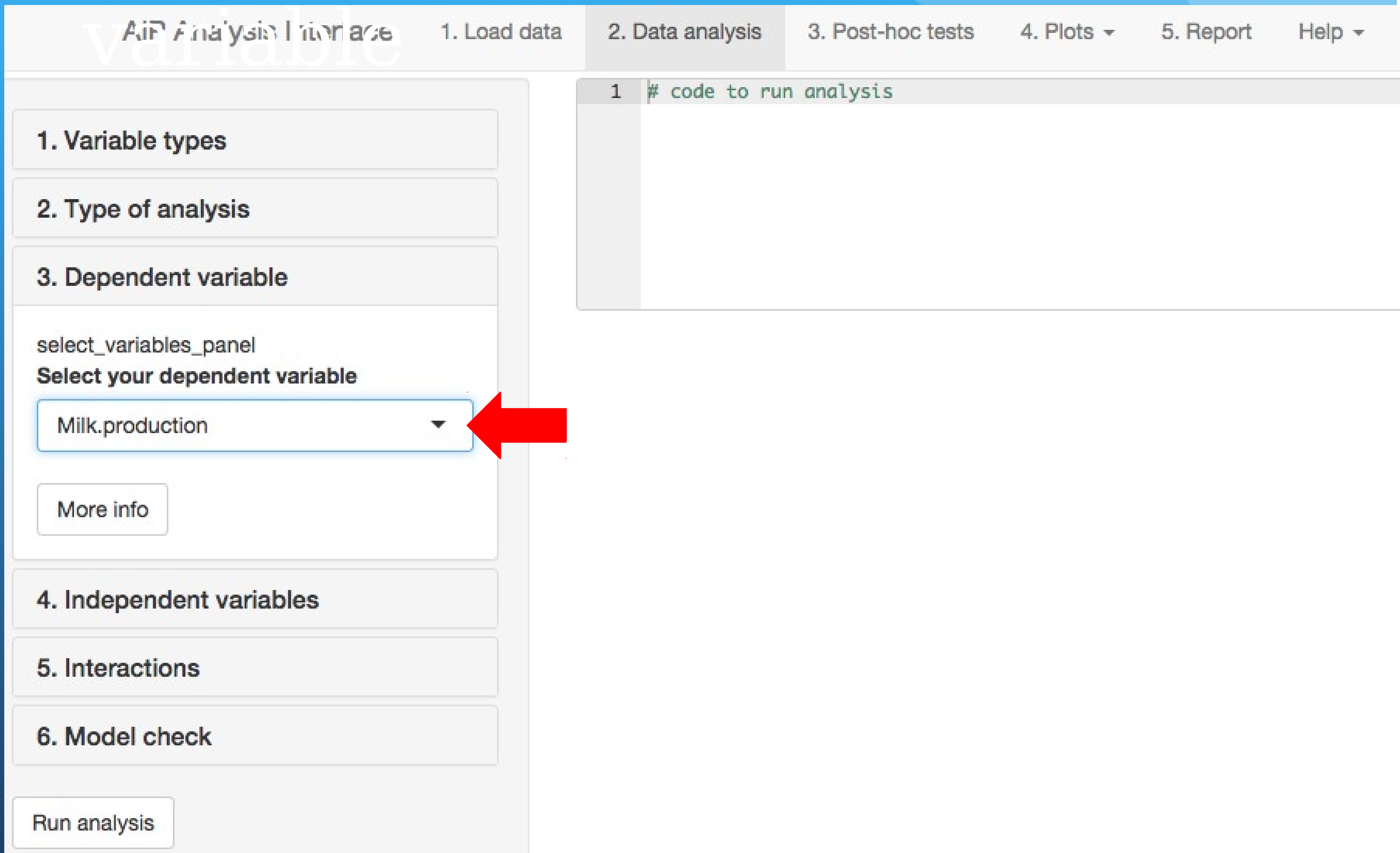


The experimental design for the milk dataset was a Complete Randomized Design Choose ANOVA

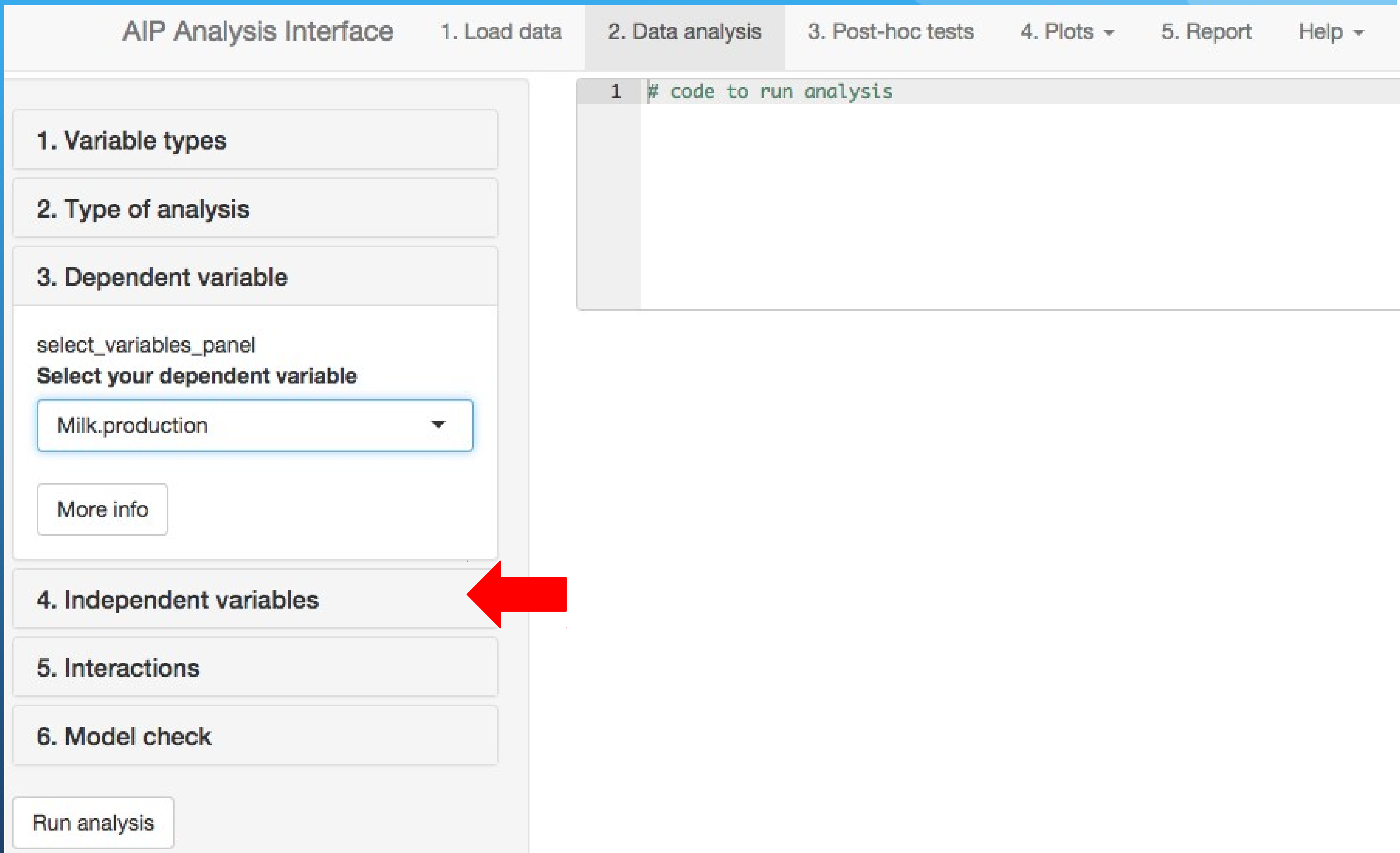
7. Click Dependent variable



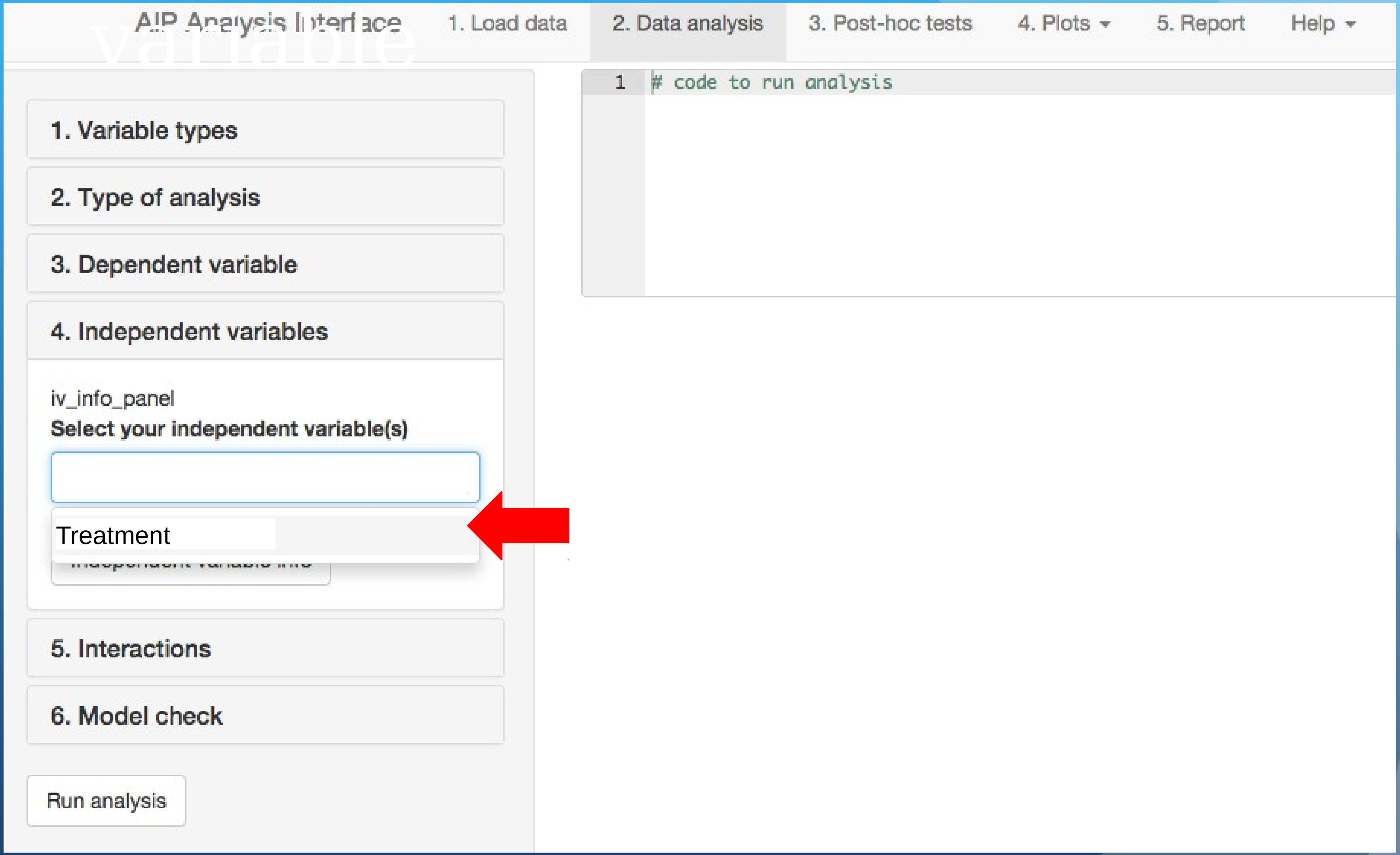
8. Select your dependent variable



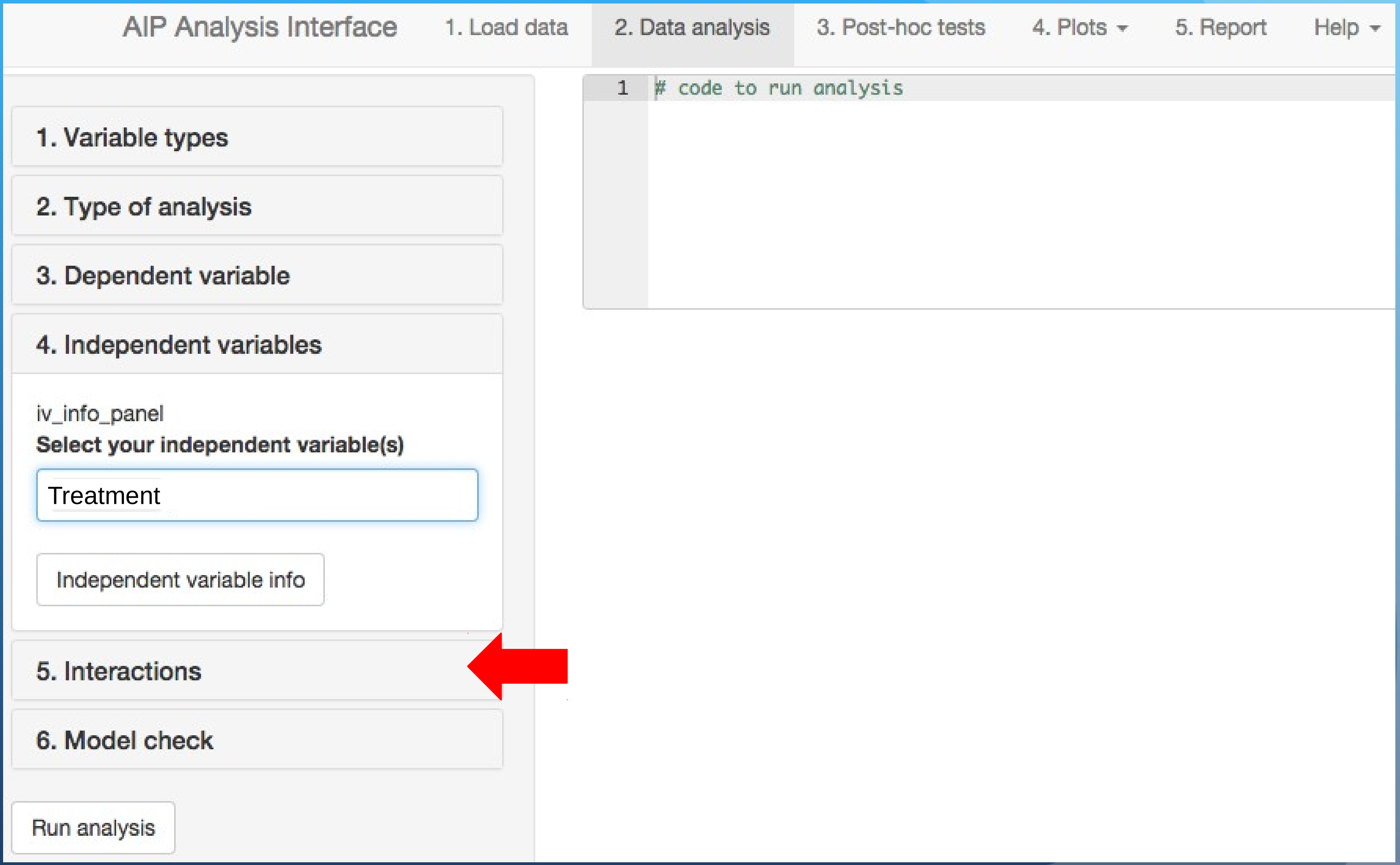
9. Click Independent variables



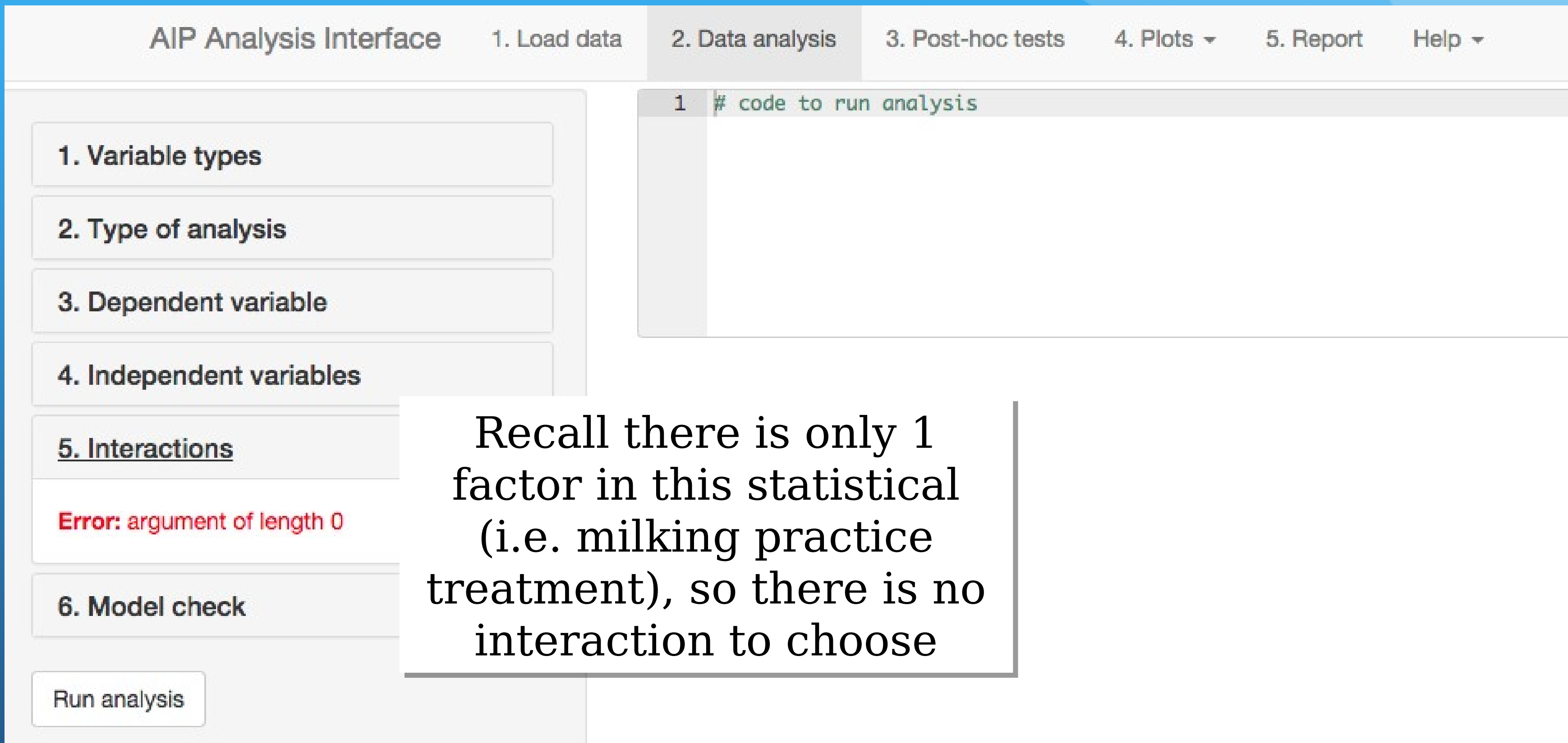
10. Select your independent variable



11. Click interactions

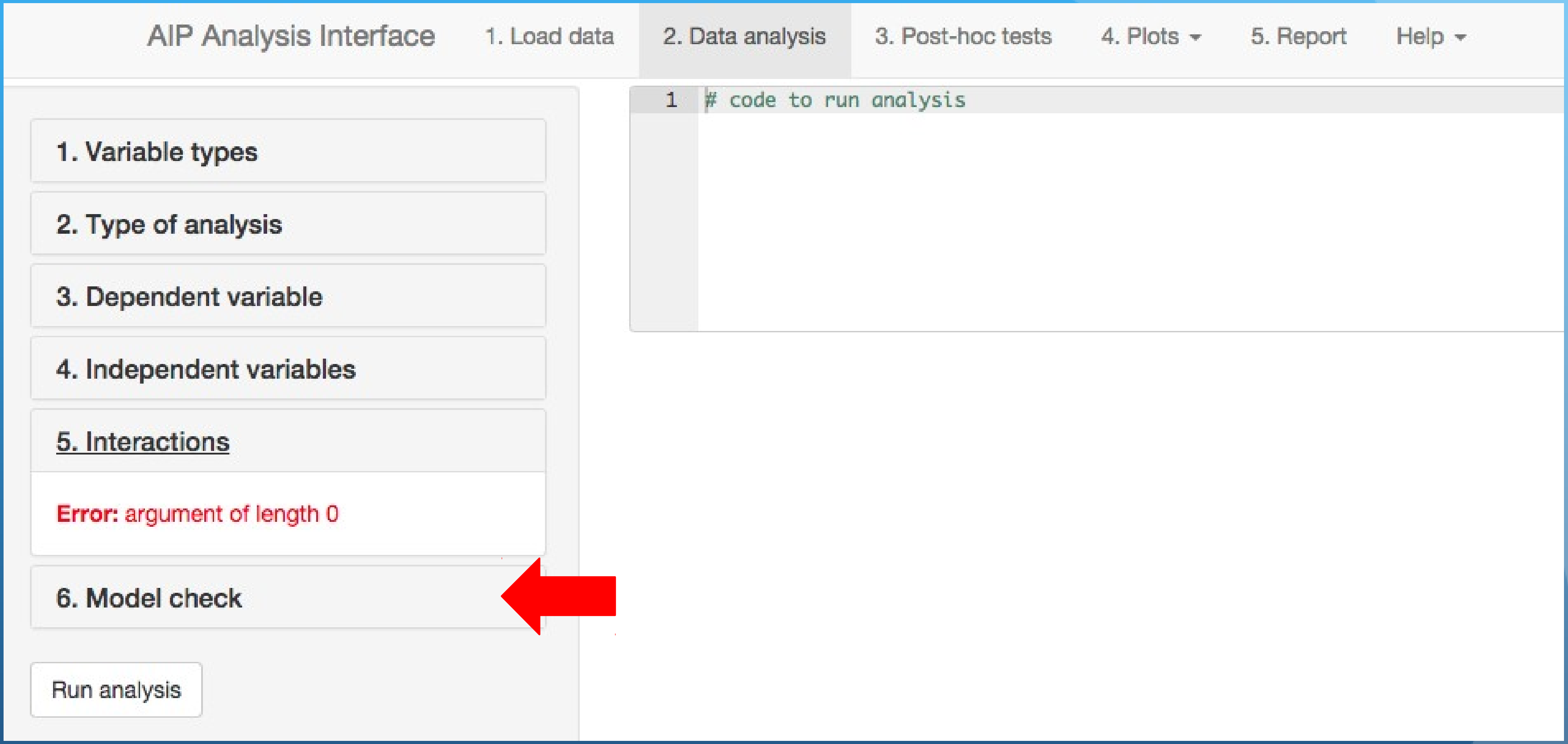


11. Click interactions

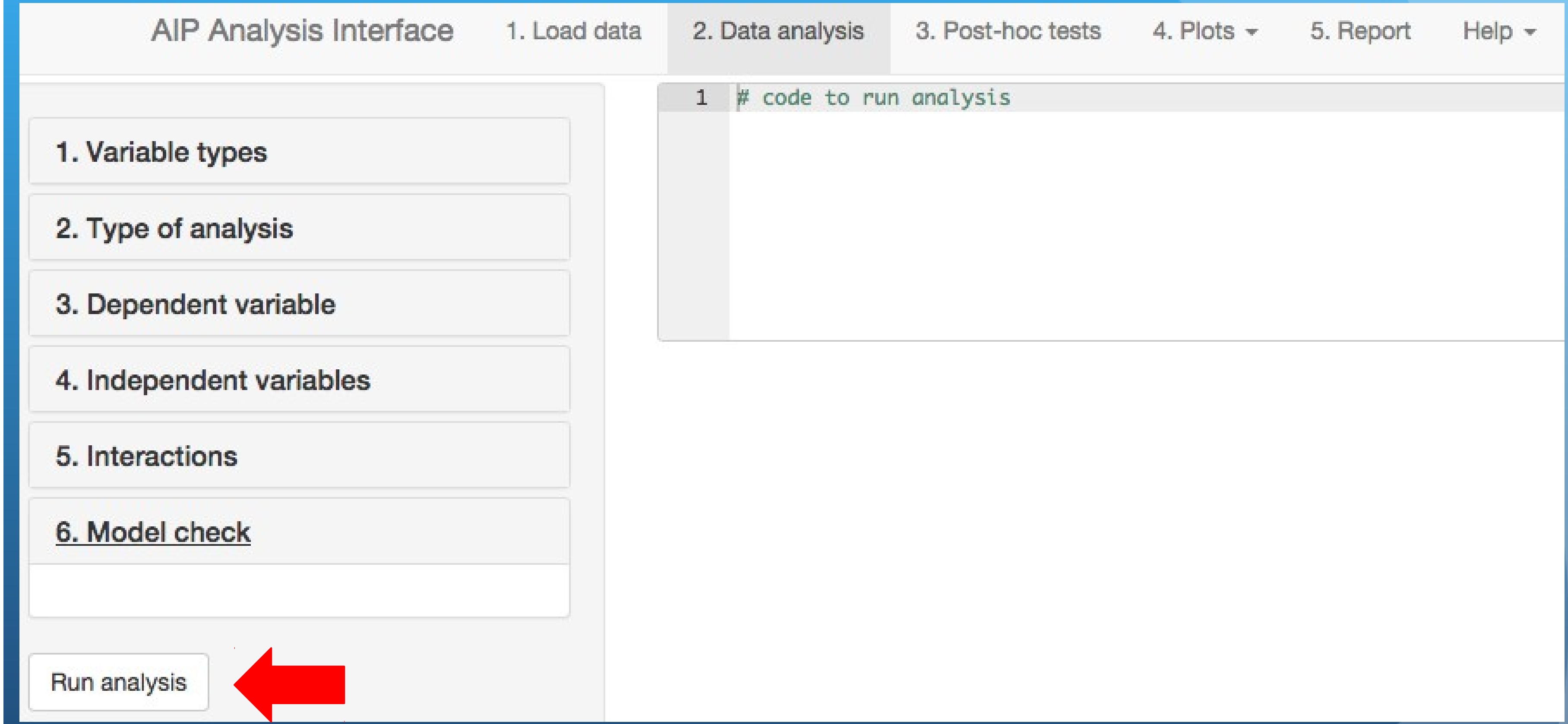


Recall there is only 1 factor in this statistical (i.e. milking practice treatment), so there is no interaction to choose

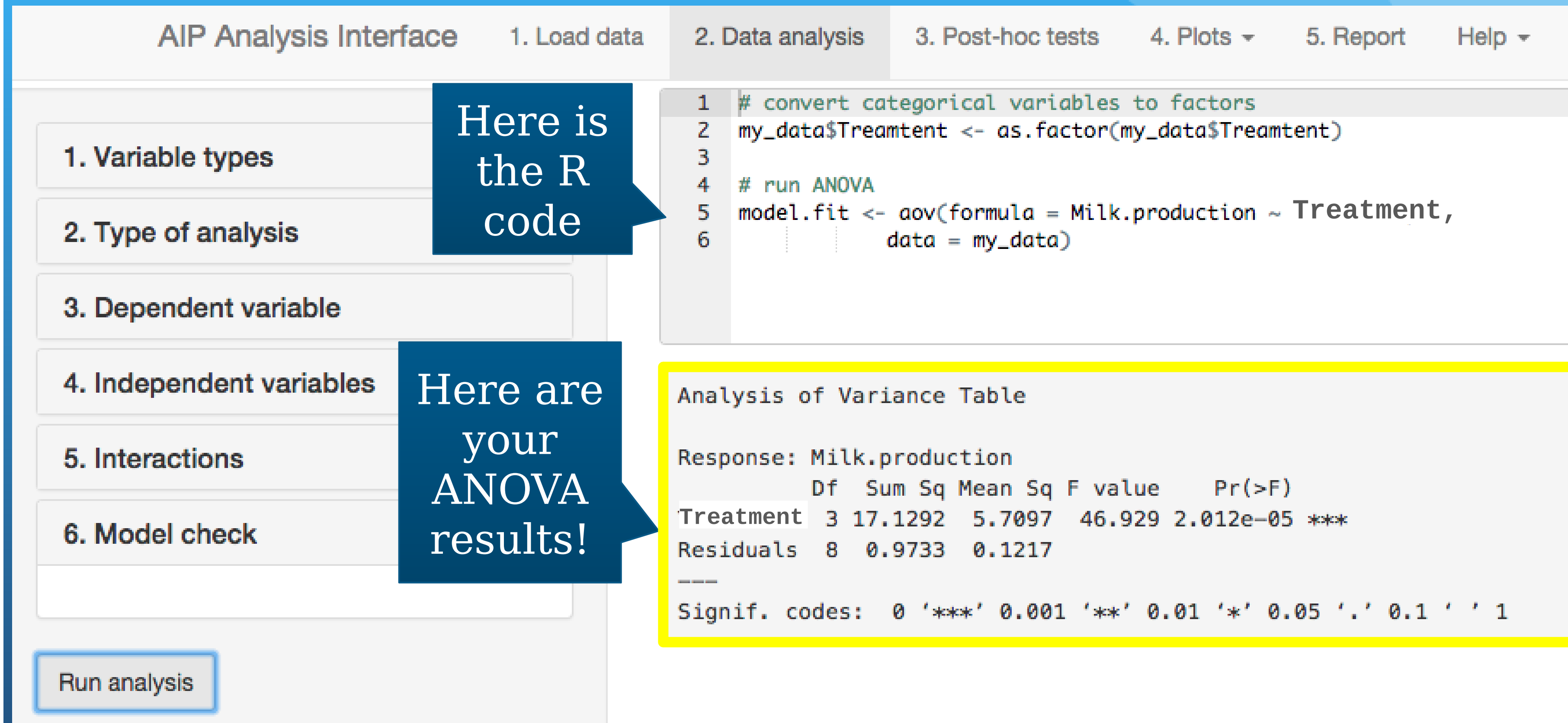
12. Click Model check



13. Click Run analysis



13. Click Run analysis



Same as our calculations

ANOVA table

Response (Y_{ij}) = milk production

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Sq (MS)	F value	P value (Pr(>p))
Total	11	18.1025			
Milking practice	3	17.1292	5.7097	46.929	<0.001***
Residual	8	0.9733	0.1217		

```
Analysis of Variance Table

Response: Milk.production
          Df  Sum Sq Mean Sq F value    Pr(>F)
Treatment  3 17.1292  5.7097  46.929 2.012e-05 ***
Residuals  8  0.9733  0.1217
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Note the significance level for our p-value

ANOVA table

Response (Y_{ij}) = milk production

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---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

*** = Significant at the p<0.001 level

Now let's use the App to determine which milking practices are significantly different, and display the results using the plot function

- [will add slides of screenshots and explanations once app is ready]

Section 2

You should now be able to:

1. Use the AIP App to analyze a CRD, including:
   - Format and load CSV file
   - Customize data analysis for CRD with a single treatment
   - Run an ANOVA and multiple mean comparison using LSD
   - Display results using the Plots tab
2. Interpret results

Section 3 Learning Objectives

Have basic understanding of:

1. Randomized Complete Block Design (RCBD)
   - Reasons to block
   - How to block and randomize treatments
   - Statistical model for RCBD
2. How to analyze data, display results, and interpret results from an RCBD experiment using the AIP App

Research question: Is there a difference in yield among 4 new wheat varieties?

What is the null hypothesis? H_0: all varieties have equal mean yield

What is the alternative hypothesis? H_A: at least two varieties differ in mean yield

What is the independent variable? Wheat variety

What is the dependent variable? Yield

Research question: Is there a difference in yield among 4 new wheat varieties?

To test H_0 using 4 replications in a field where there is a known gradient in soil nitrogen (shown below), what would you choose as:
(1) the experimental design (identify the experimental unit)
(2) the layout/orientation of your plots?
And (3) how would you assign treatments to plots?

Experimental Field

Discuss and draw the layout in groups

(low) → (high)

Soil Nitrogen

Research question: Is there a difference in yield among 4 new wheat varieties?

1. What is the experimental design and experimental unit?

Experimental Field

1	**2**	**3**	**4**
5	6	7	8
9	10	11	12
13	14	15	16

Randomized Complete Block Design (RCBD) with 4 trts and 4 blocks.
4 trts x 4 blocks = 16 plots

Plots are called **EXPERIMENTAL UNITS**, defined as the “object” that the treatments are applied to.

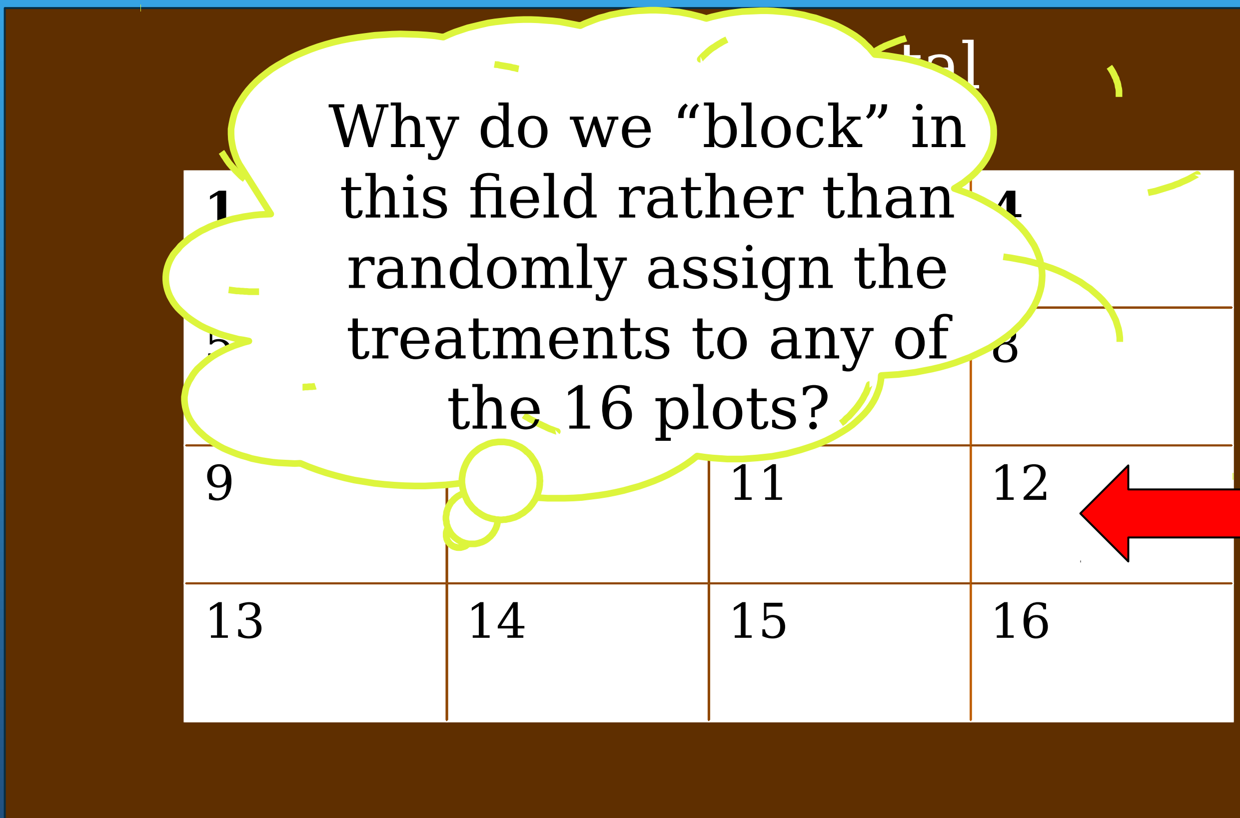
(low)

(high)

Soil Nitrogen

Research question: Is there a difference in yield among 4 new wheat varieties?

Randomized Complete Block Design (RCBD) with 4 trts and 4 blocks.
4 trts x 4 blocks = 16 plots



Why do we “block” in this field rather than randomly assign the treatments to any of the 16 plots?

Plots are called **EXPERIMENTAL UNITS**, defined as the “object” that the treatments are applied to.

(low
)

(high)

Soil Nitrogen

Research question: Is there a difference in yield among 4 new wheat varieties?

2. How do we arrange the blocks in the field?

Experimental Field

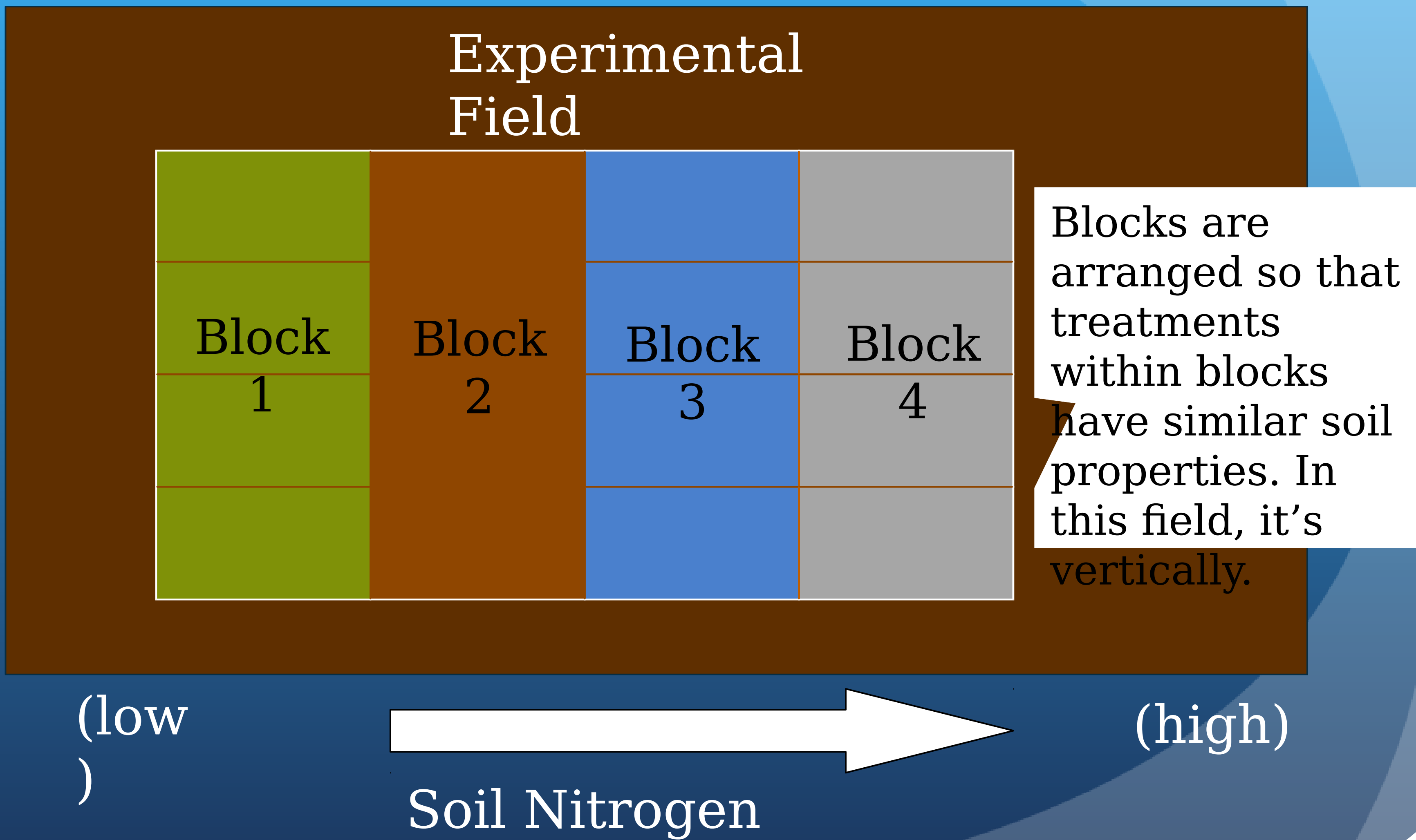
1	**2**	**3**	**4**
5	6	7	8
9	10	11	12
13	14	15	16

(low) → (high)

Soil Nitrogen

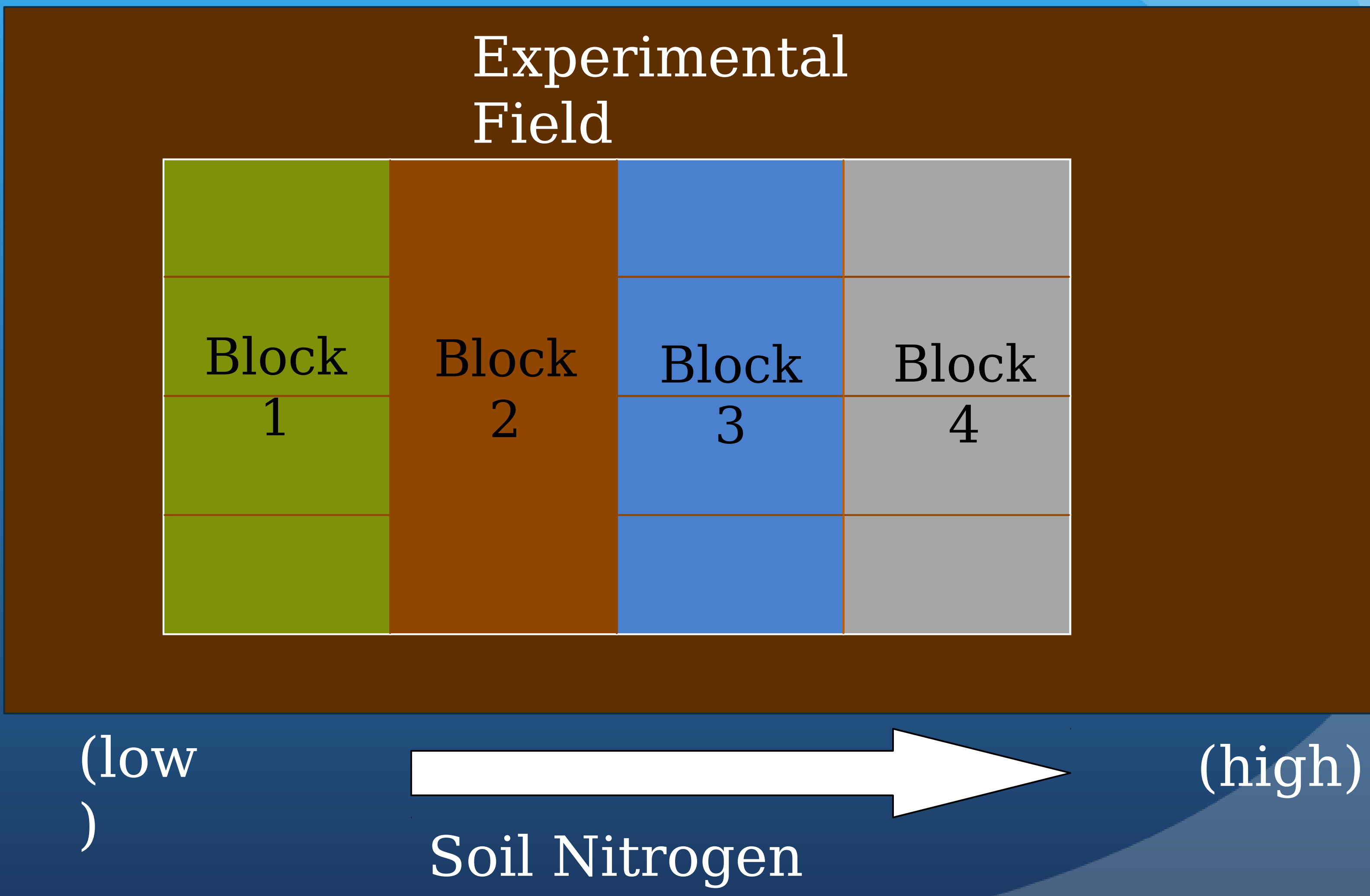
Research question: Is there a difference in yield among 4 new wheat varieties?

2. How do we arrange the blocks in the field?

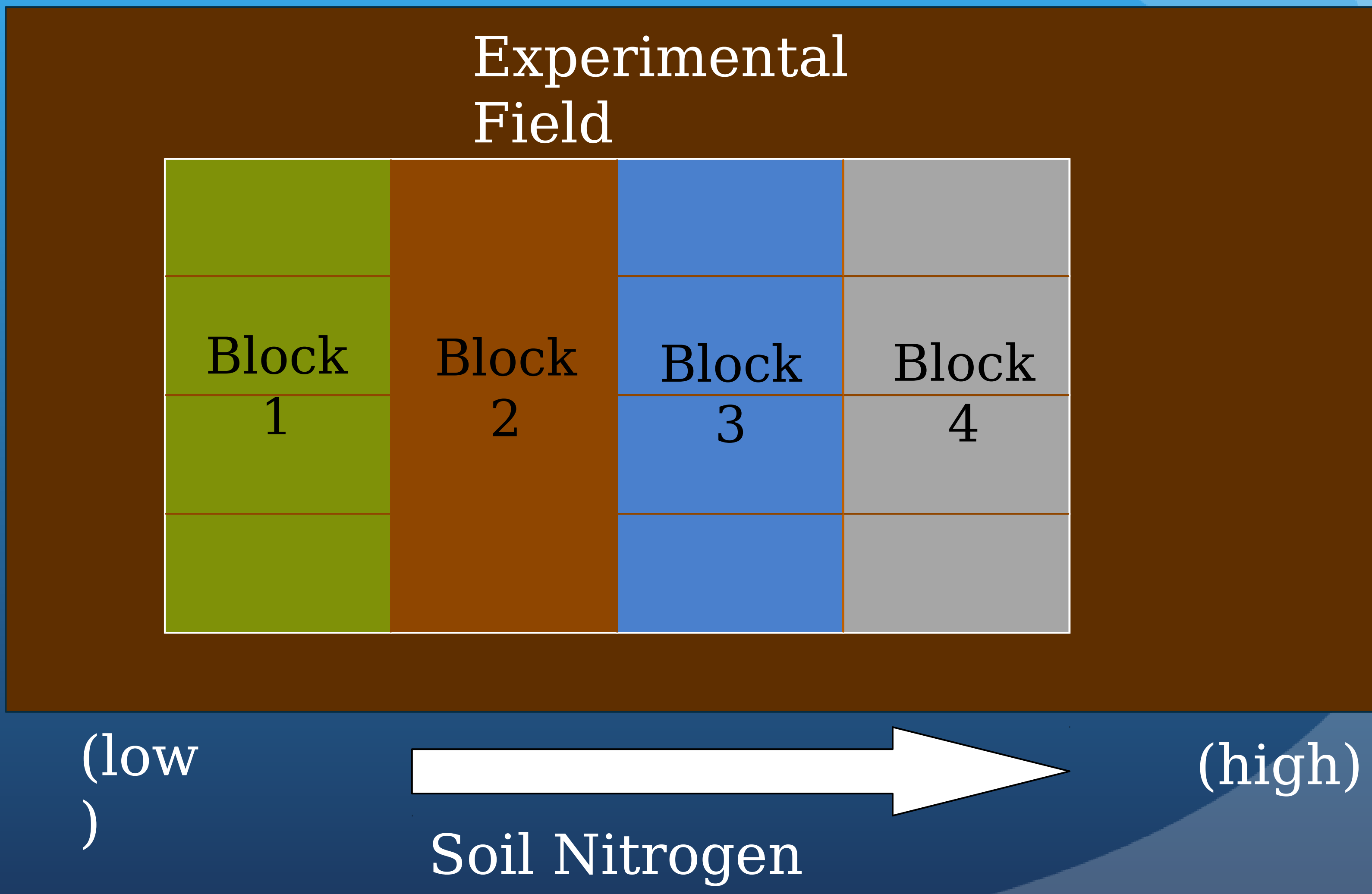


Research question: Is there a difference in yield among 4 new wheat varieties?

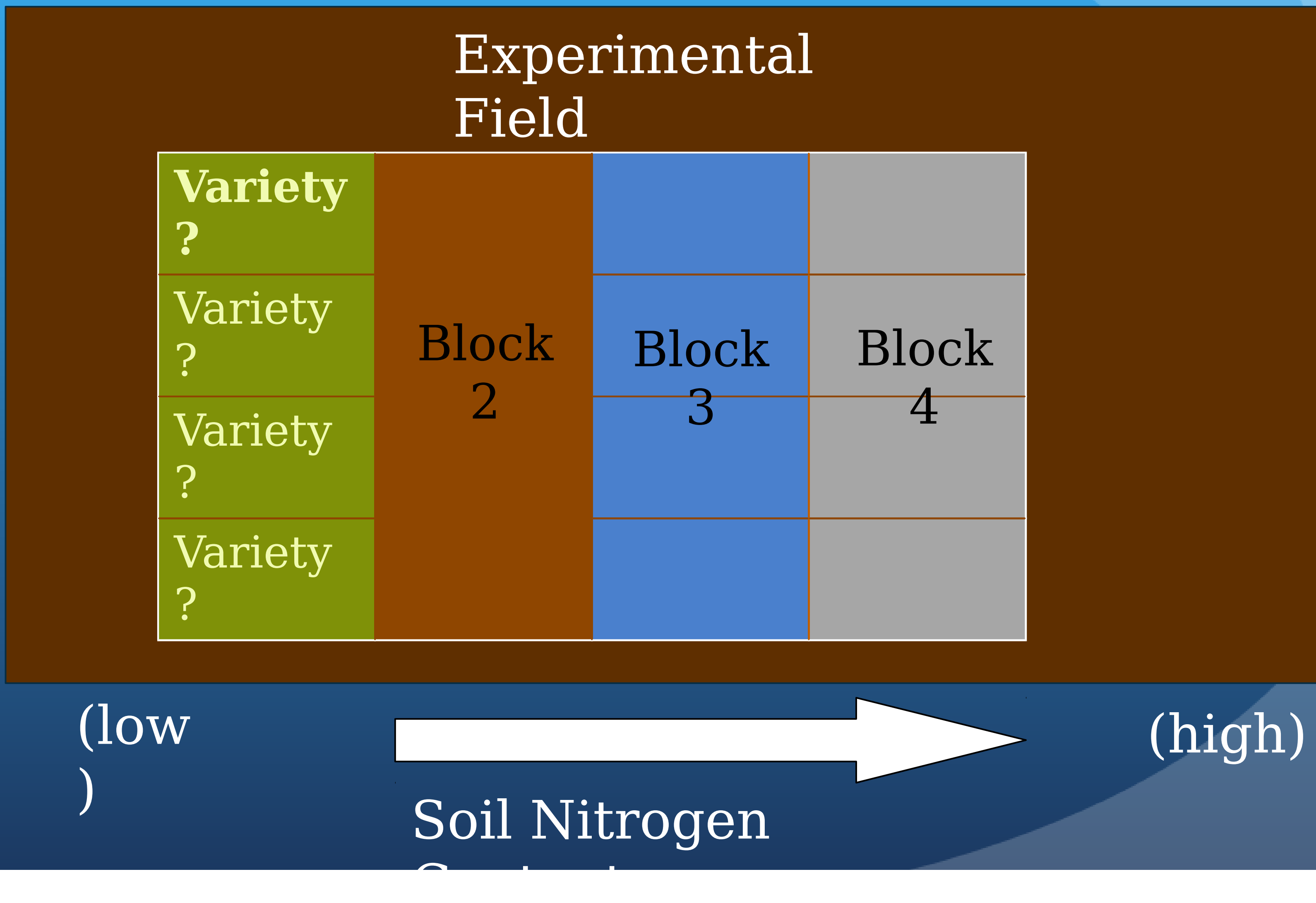
3. How are treatments assigned to plots?



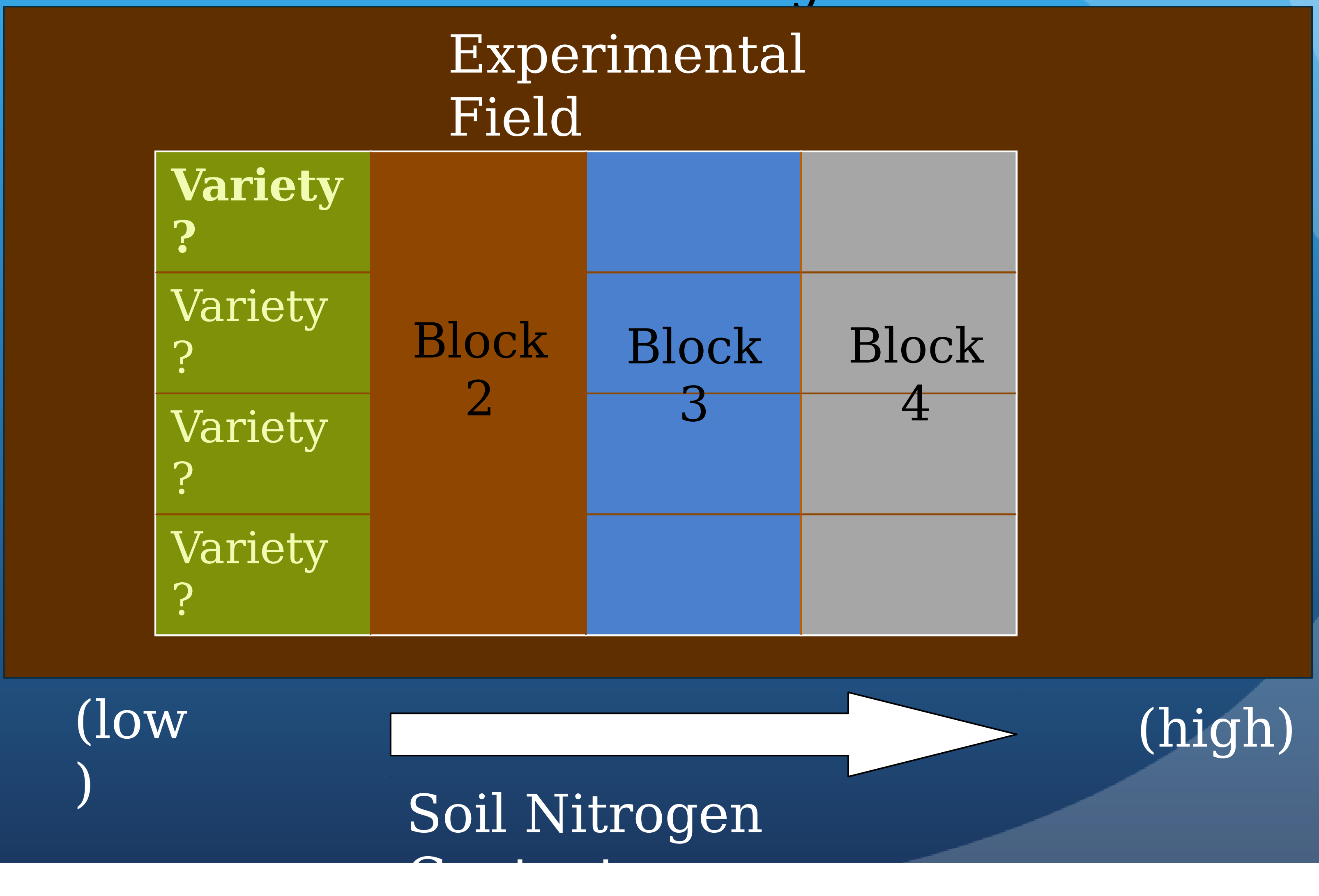
All treatments are **RANDOMLY** assigned to plots within each block.



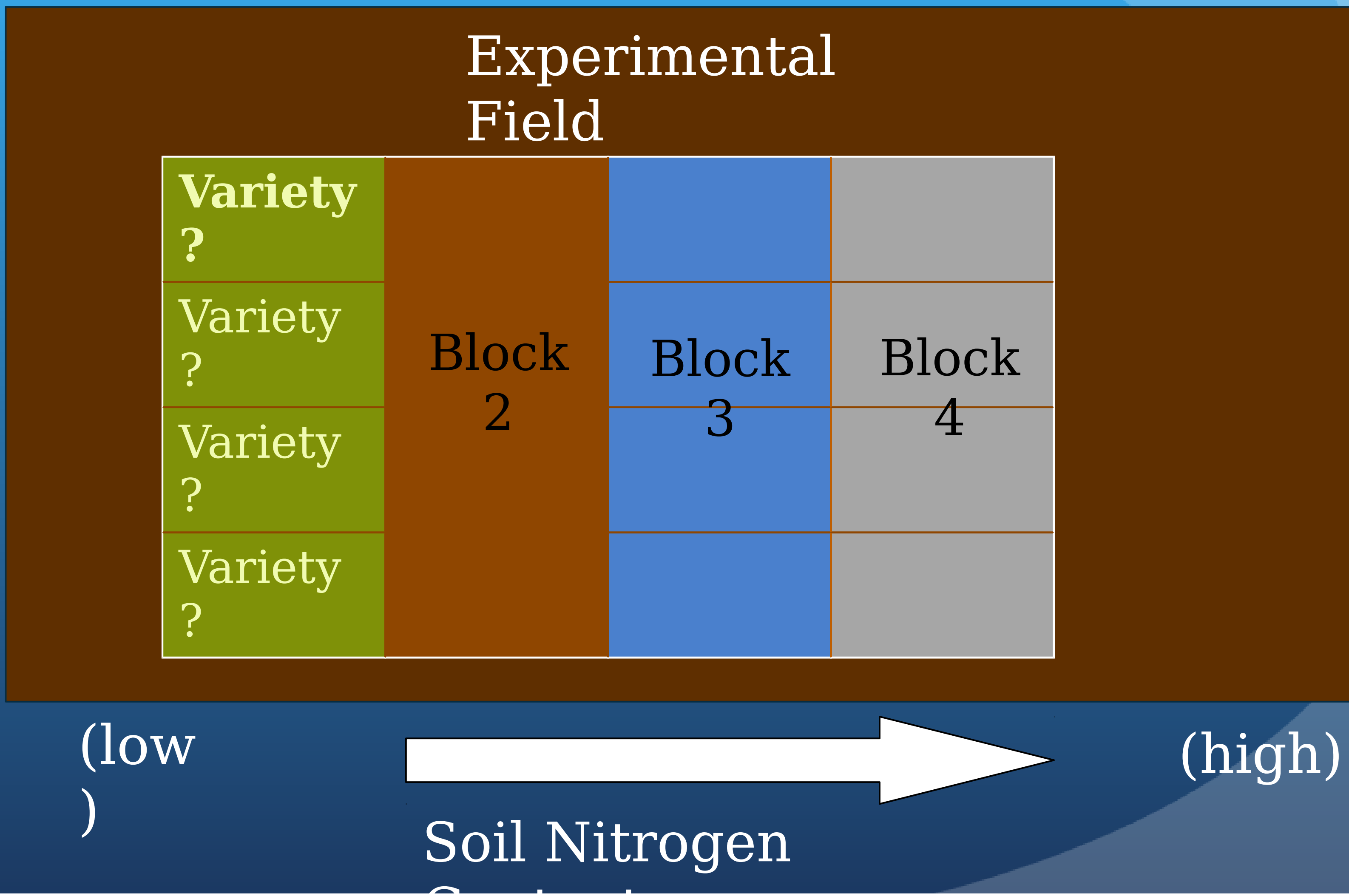
Starting with block 1, randomly assign the 4 wheat varieties to the 4 plots.

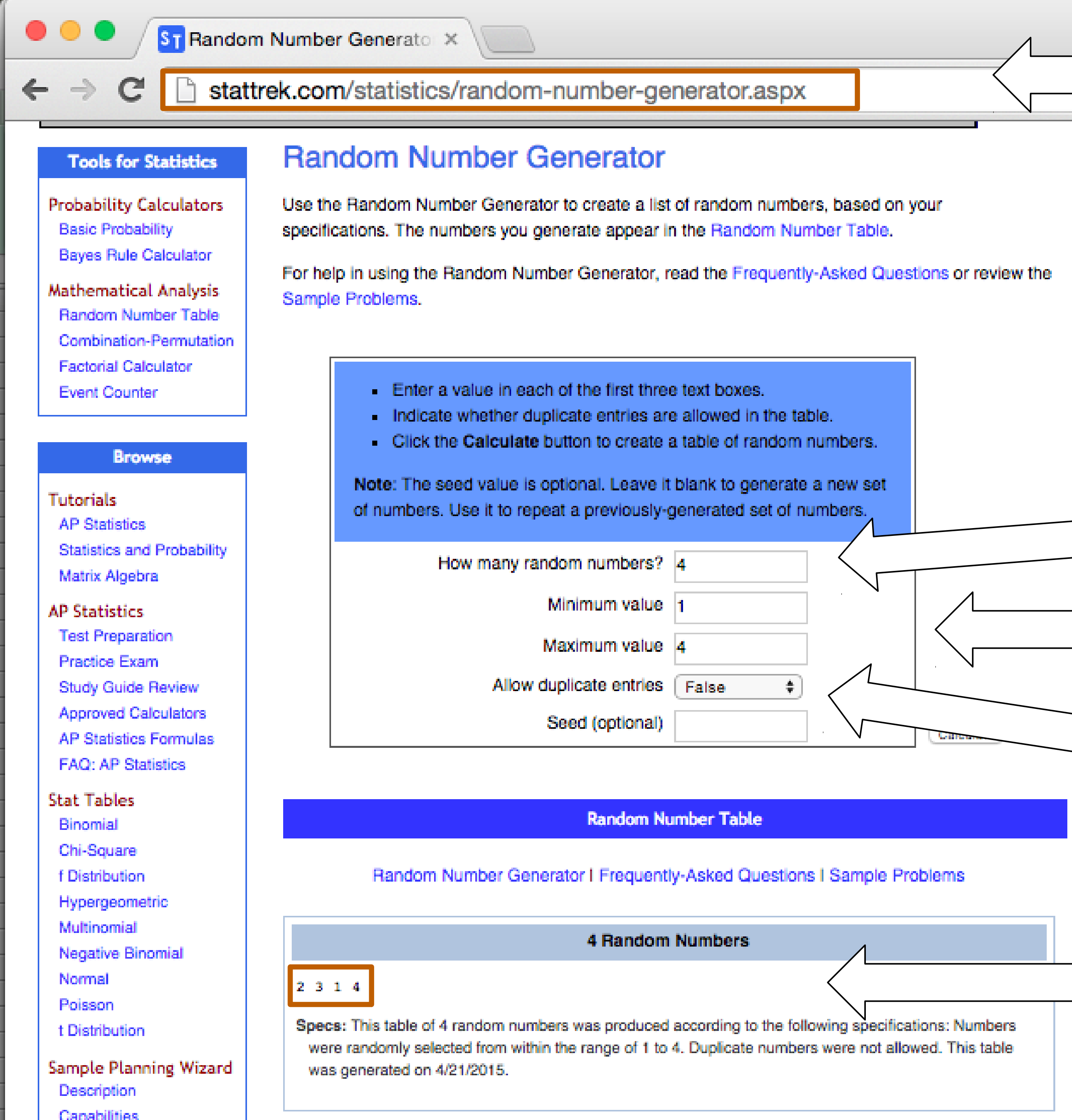


But how do we do it randomly?



Using a random number generator, or drawing numbers from a hat without replacement. Any other ideas?





Example of free random number generator online

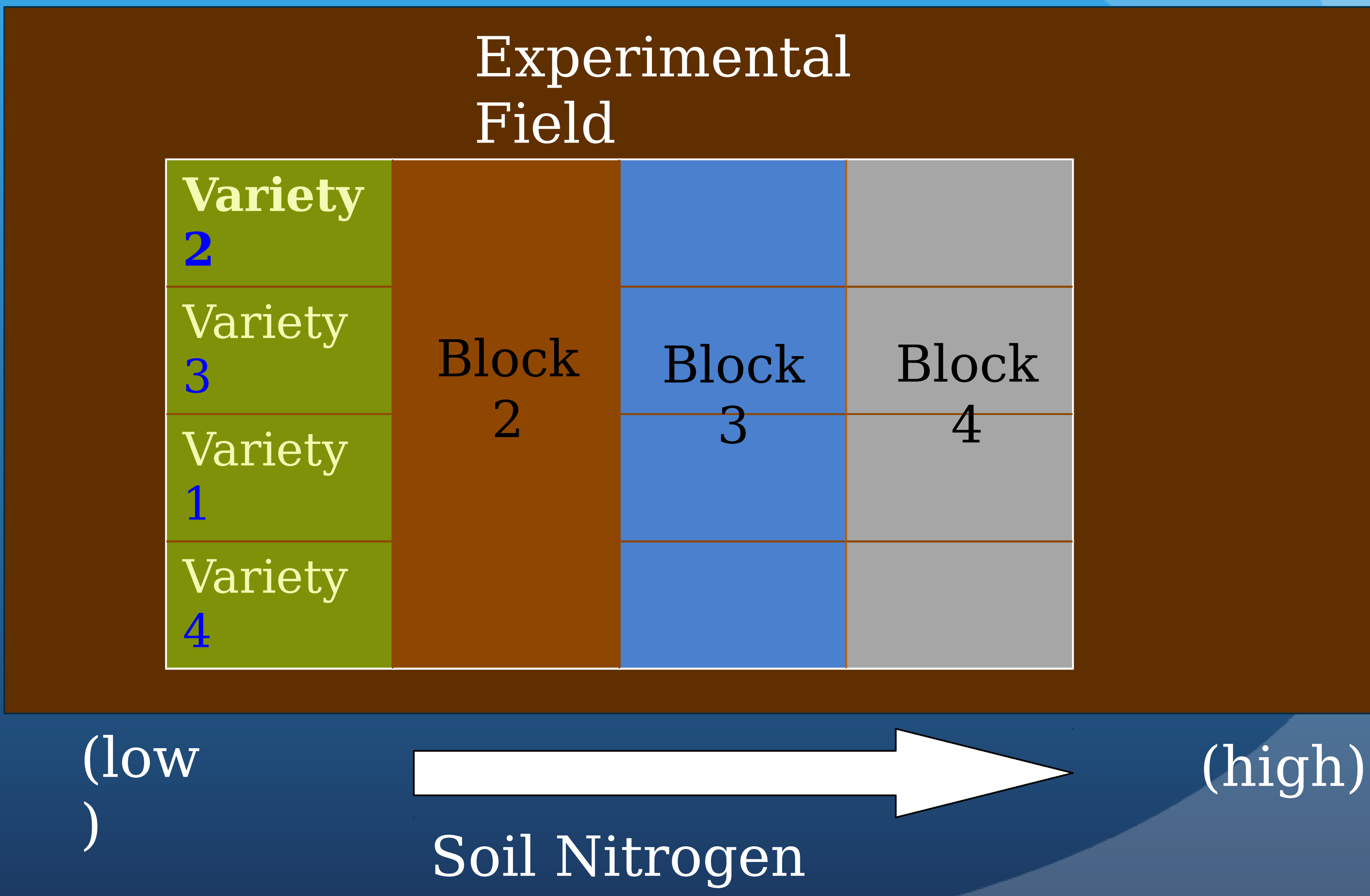
Enter number of treatments (4 varieties)

And the min and max values (1

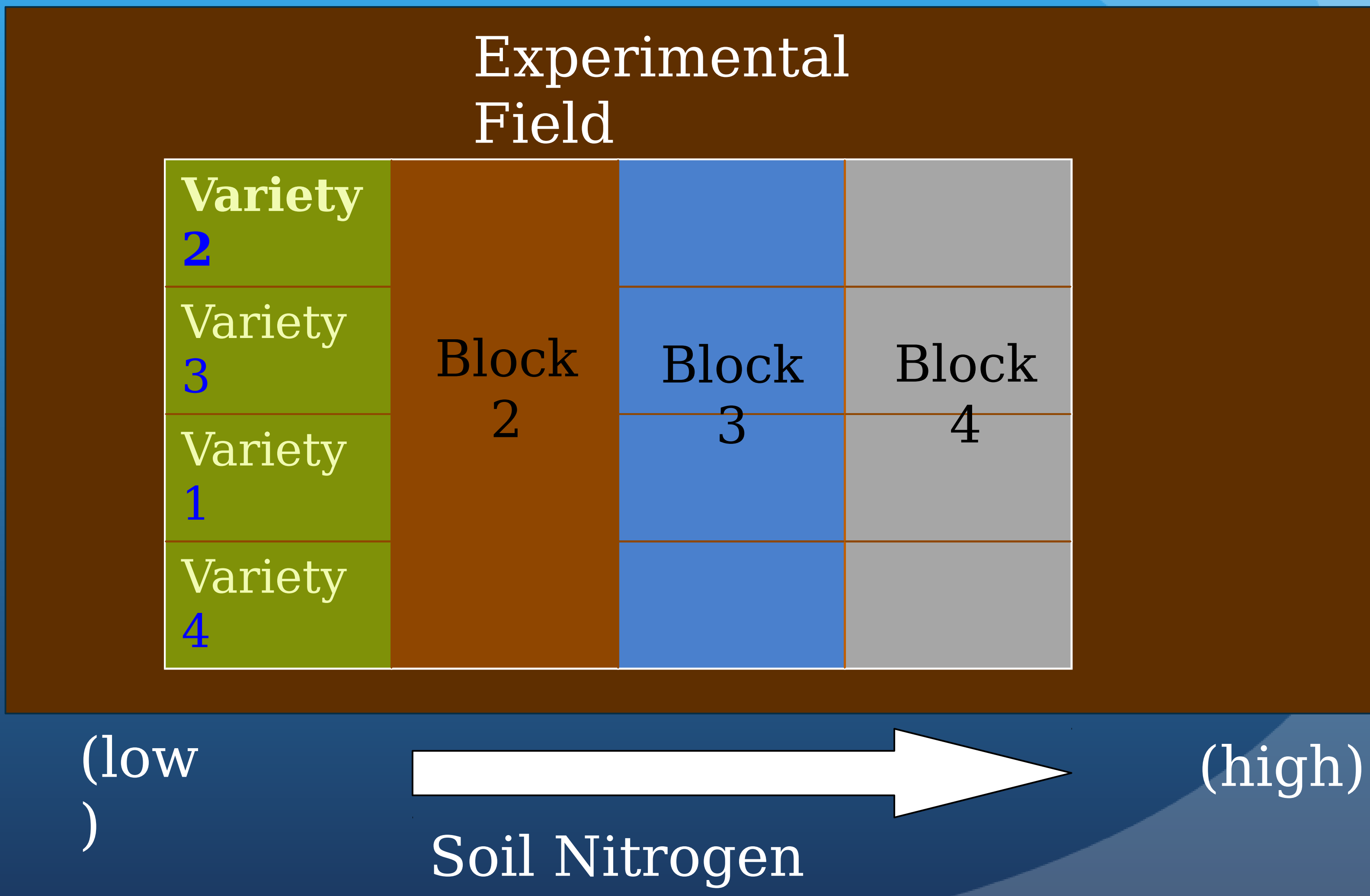
Choose "False" for duplicate entries so you only get 1, 2 ,3 and 4 as possibilities.

The order of your treatments for block 1! This time it was

Randomly assign treatments to block 1!



Repeat for each block by refreshing website, or by replacing numbers into hat and drawing them again for each block.



Repeat for each block by refreshing website, or by replacing numbers into hat and drawing them again for each block.

Experimental Field

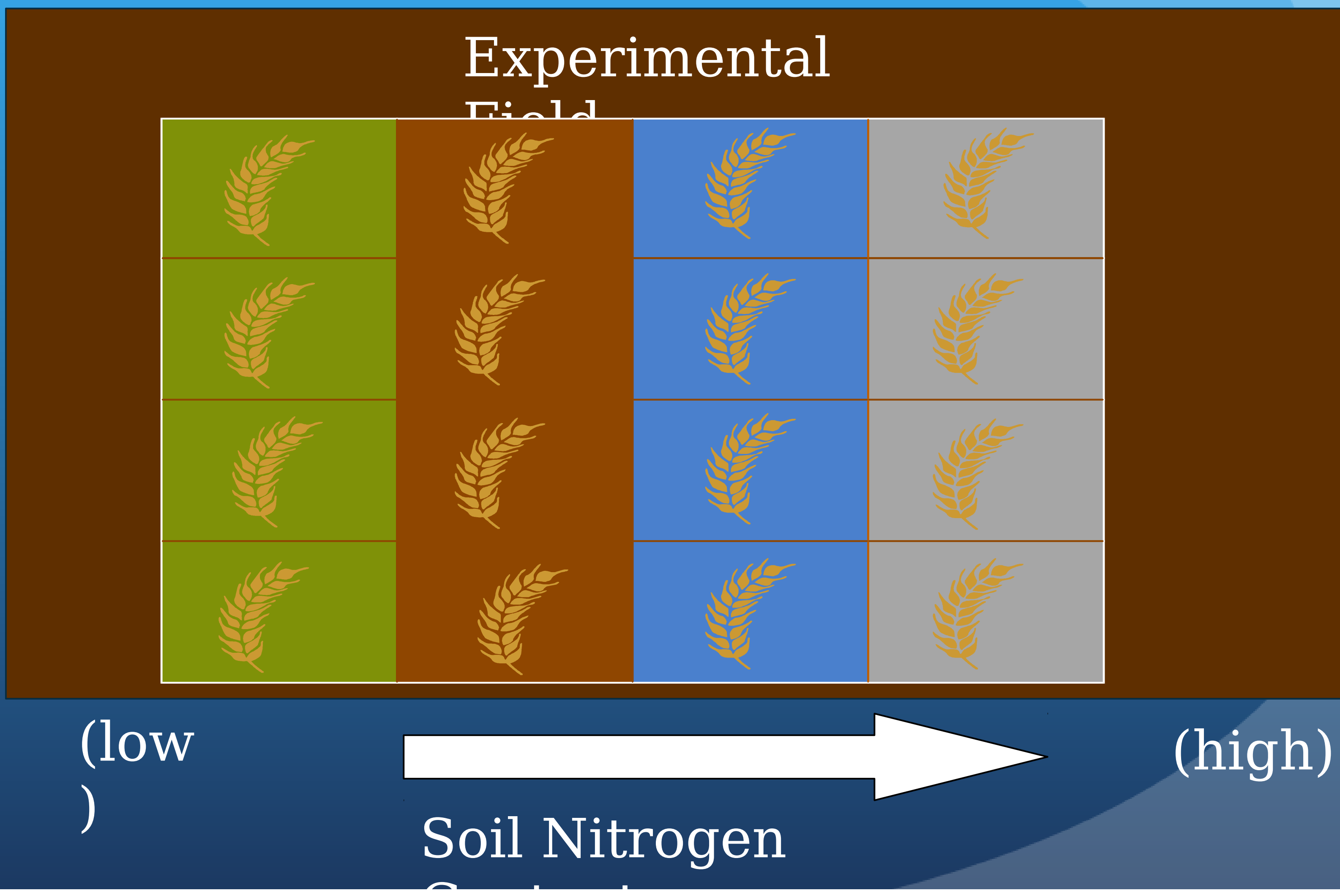
For example,

Variety 2	**Variety 1**	**Variety 3**	**Variety 2**
Variety 3	Variety 3	Variety 4	Variety 1
Variety 1	Variety 4	Variety 1	Variety 3
Variety 4	Variety 2	Variety 2	Variety 4

(low) → (high)

Soil Nitrogen Content

Our field trial is underway!



Let’s review, why use blocking variable?

- Blocking is done when the experimental units (plot, animal, potted plant, etc.) **are not uniform** due to some naturally occurring differences prior to applying a treatment.

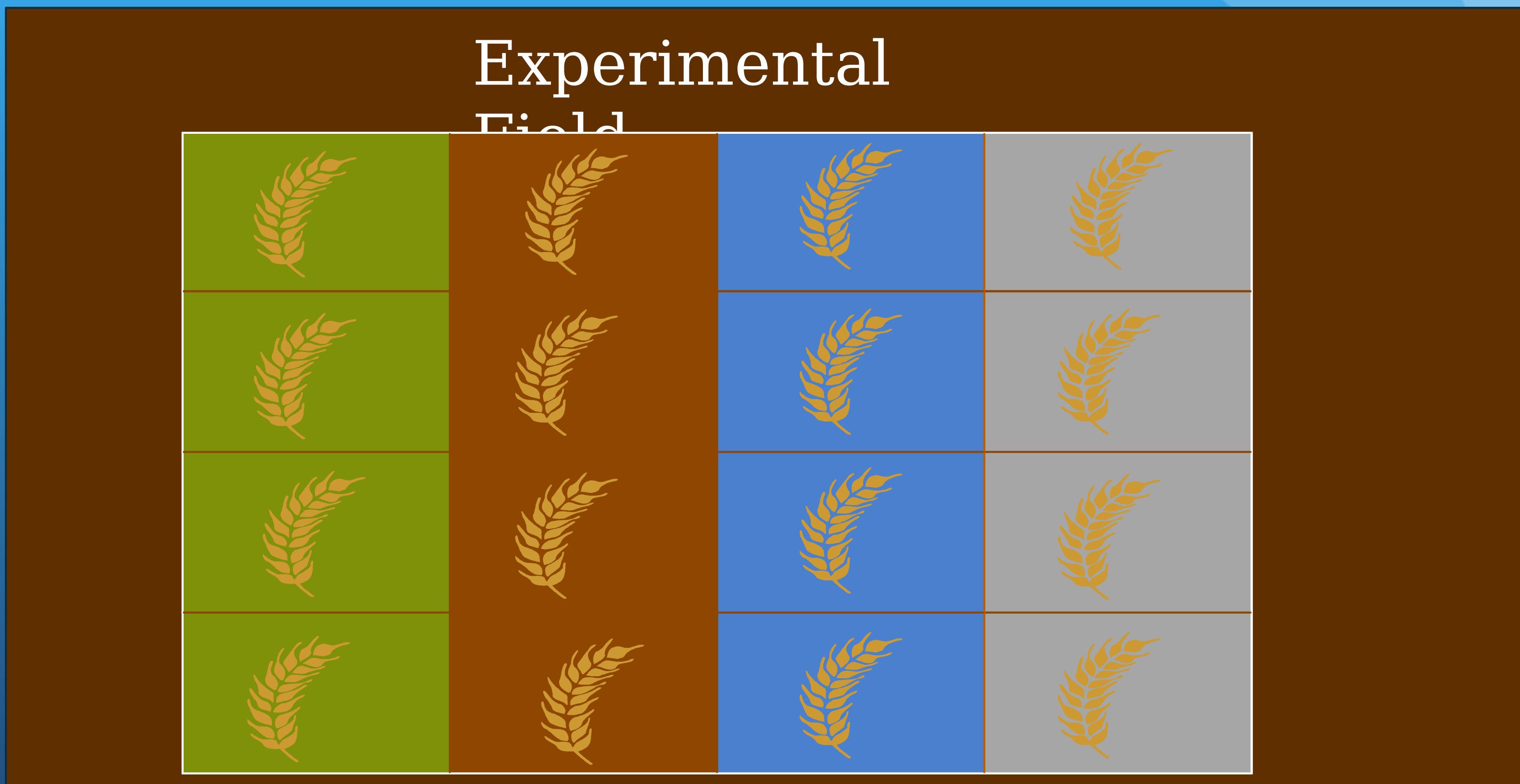
Examples??

- A sloping agricultural field where plots are the experimental unit and the upper, middle, and lower hill-slope are the blocks.
- Multiple cattle ranches where the cows are the experimental unit, and the block is the cattle ranch.

- Blocks can account for differences that may affect the dependent variable, and therefore reduce unexplained variation (error), which improves our ability to detect differences among treatments!

What statistical model predicts yield for an individual plot in this experiment?

$\mathbf{Y_{ij} = ?}$ where i is ith treatment, and j is the jth block



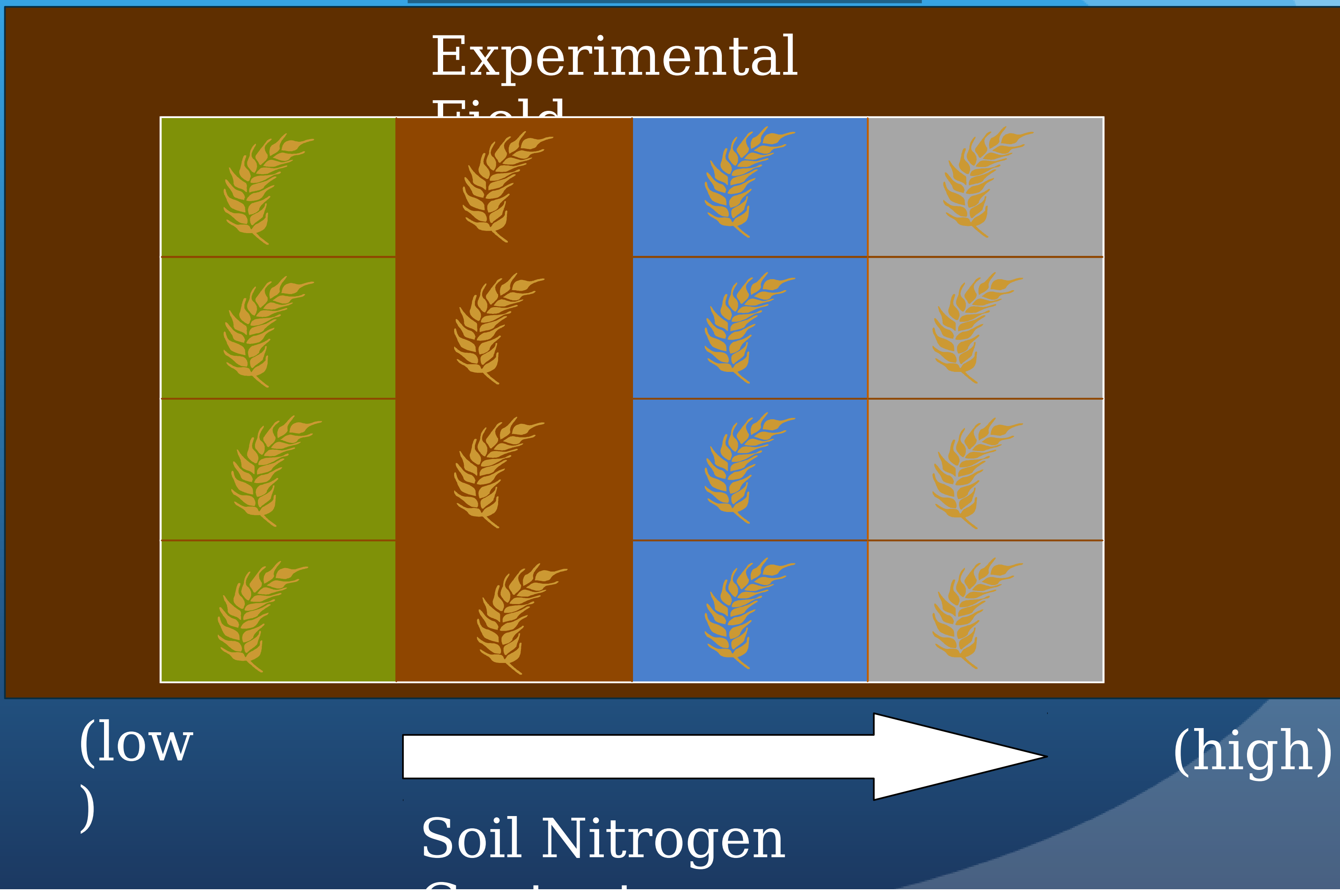
(low)

(high)

Soil Nitrogen

What statistical model predicts yield for an individual plot in this experiment?

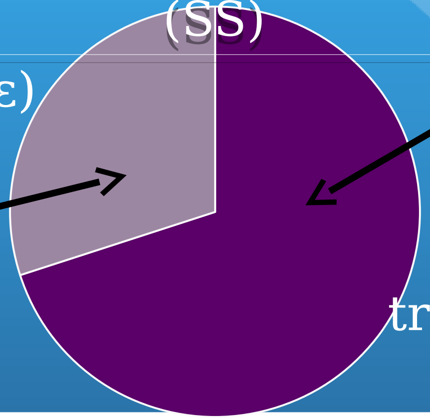
$$Y_{ij} = \mu + \tau_i + \nu_j + \varepsilon_{ij}$$



Recall

Complete Randomized Design (CRD):

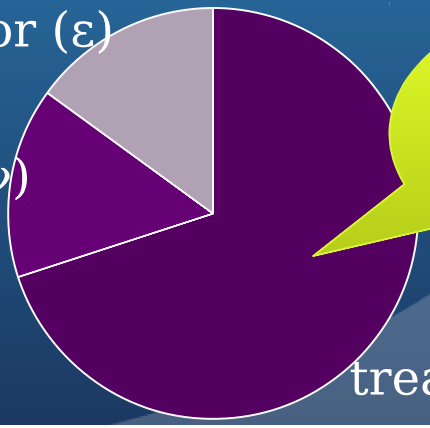
$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$



If we can explain more of the variation than 'treatment' alone, add it to the experimental design and to the model lower the error increase power!

Randomized Complete Block Design (RCBD):

$$Y_{ij} = \mu + \tau_i + \nu_j + \varepsilon_{ij}$$



Exercise 2

In groups, analyze the ‘wheat variety’ dataset using the AIP App:

1. Determine if there is an effect of wheat variety on yield, and if so, what are the relative differences among the wheat varieties? Use Protected LSD.

2. Write main conclusions using biological and statistical interpretations.

- [Finished up to this point…
- Will add screenshots once app is ready: loading RCBD wheat data, running ANOVA, LSD mean separation, display results]

Other resources for statistical software:

- Free (coding): R
  - Download for Windows: https://cran.r-project.org/bin/windows/base/
  - Download for Mac: https://cran.r-project.org/bin/macosx/
- Cost (GUIs): JMP, Sigma Plot (graphics)