

Gaussian distributions

QQ plots

t-tests

Comparing two datasets

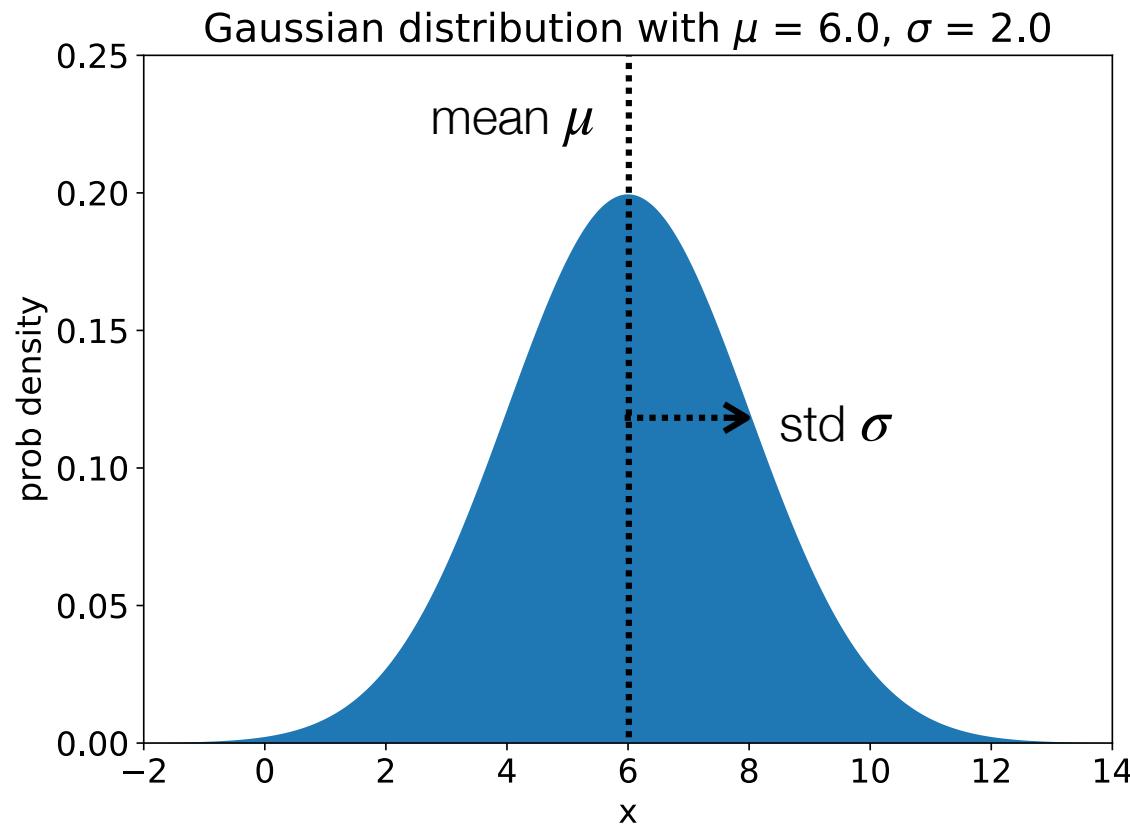


Practical Statistics for Experimental Biologists
Lecture 3
Wednesday, 29 July 2020
10:00am - 12:00pm

Gaussian distributions

The normal distribution is ubiquitous in statistics

“Gaussian distribution” = “normal distribution”



$x \sim \text{Normal}(\mu, \sigma^2)$

drawn from

mean

variance

The central limit theorem makes the normal distribution extremely relevant

The average of multiple random variables is approximately normally distributed, (mostly) regardless of the distribution each is drawn from.

$$x_1 \sim p_1(x)$$

$$x_2 \sim p_2(x)$$

...

$$x_N \sim p_N(x)$$

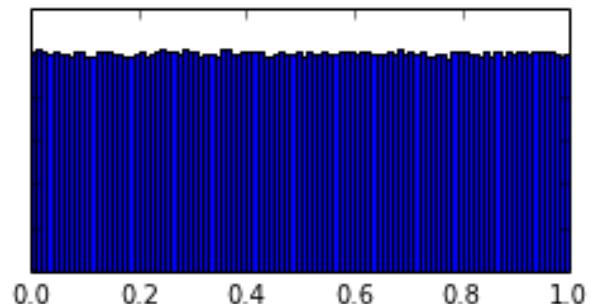
$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_N}{N} \quad \rightarrow \quad \bar{x} \sim \text{Normal}(\mu, \sigma^2)$$

This means that, if many sources additively contribute to an experimental measurement, independent measurements will be approximately normally distributed.

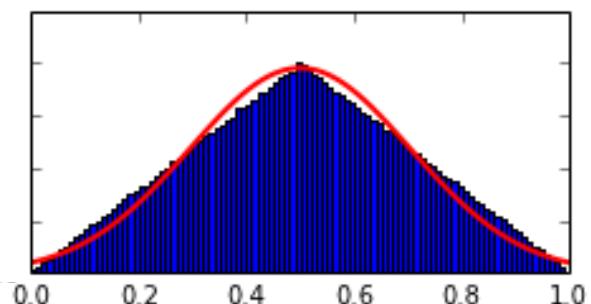
This is why statisticians so often assume that experimental measurements follow normal distributions.

Suppose x_1, x_2, \dots, x_N are drawn from a uniform (i.e. flat) probability distribution that stands from 0 and 1

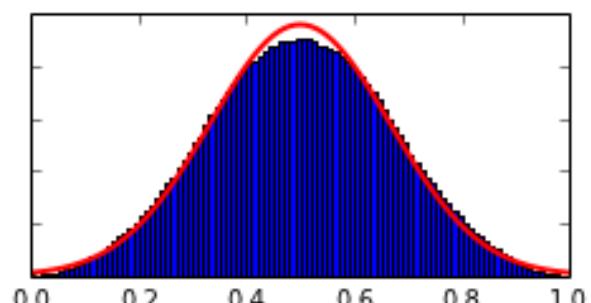
x_1



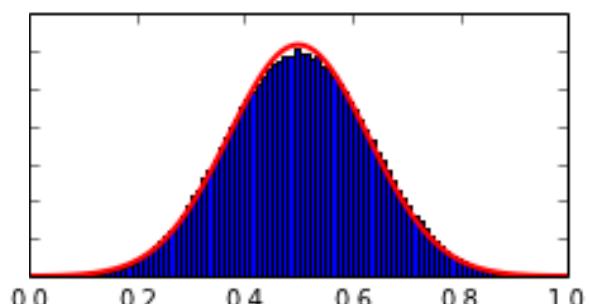
$$\frac{x_1 + x_2}{2}$$



$$\frac{x_1 + x_2 + x_3}{3}$$



$$\frac{x_1 + x_2 + x_3 + x_4 + x_5}{5}$$



The central limit theorem makes the normal distribution extremely relevant

The parameters of a statistical model that has been fit to a large dataset will have lingering uncertainty, but this uncertainty will (very often) be approximately normally distributed.

θ : model parameter inferred from data

$$\theta \sim \text{Normal}(\mu, \sigma^2)$$

Then the goal of statistical inference is to determine the mean and std of this distribution.

μ : best estimate of θ , denoted $\hat{\theta}$

σ : lingering uncertainty in θ ; affects confidence interval

Example 1: Human Sex Ratio

The human sex ratio at birth is slightly skewed towards boys rather than girls.

count	
male	484382
female	453841
total	938223



probability of male birth

estimate: 51.63%

95% CI: [51.53%, 51.73%]

Arbuthnot J (1711). An Argument for Divine Providence, taken from the Constant Regularity observed in the Births of both Sexes.

We assume the number of male babies (versus female babies) is drawn from a binomial distribution

data

$n = 484,382$: number of male births

$N = 938,223$: total number of births

model

$n \sim \text{Binom}(q, N)$

q : probity of a male birth

The assume probability distribution is called the sampling distribution

goals

1. Compute a best estimate \hat{q} for q
2. Compute a confidence interval for q

The standard estimate of probability is just the ratio of counts

$n = 484,382$: number of male births

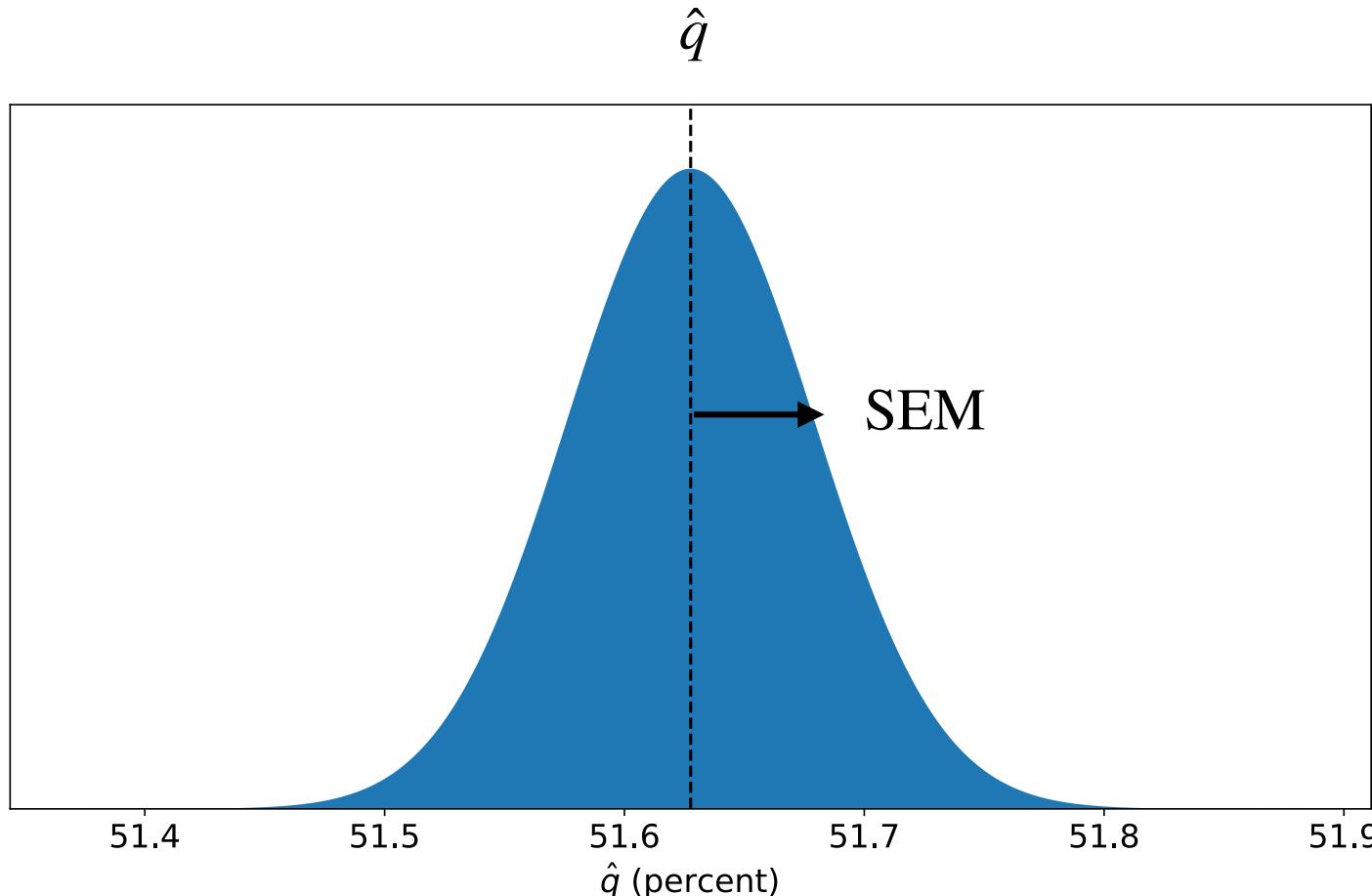
$N = 938,223$: total number of male births

$\hat{q} = \frac{n}{N} = 51.63\%$: estimated probability of a newborn being male

The lingering uncertainty in q is (verily nearly) described by a normal distribution centered on the estimate \hat{q} .

The standard deviation of this distribution is called the standard error of the mean (SEM).

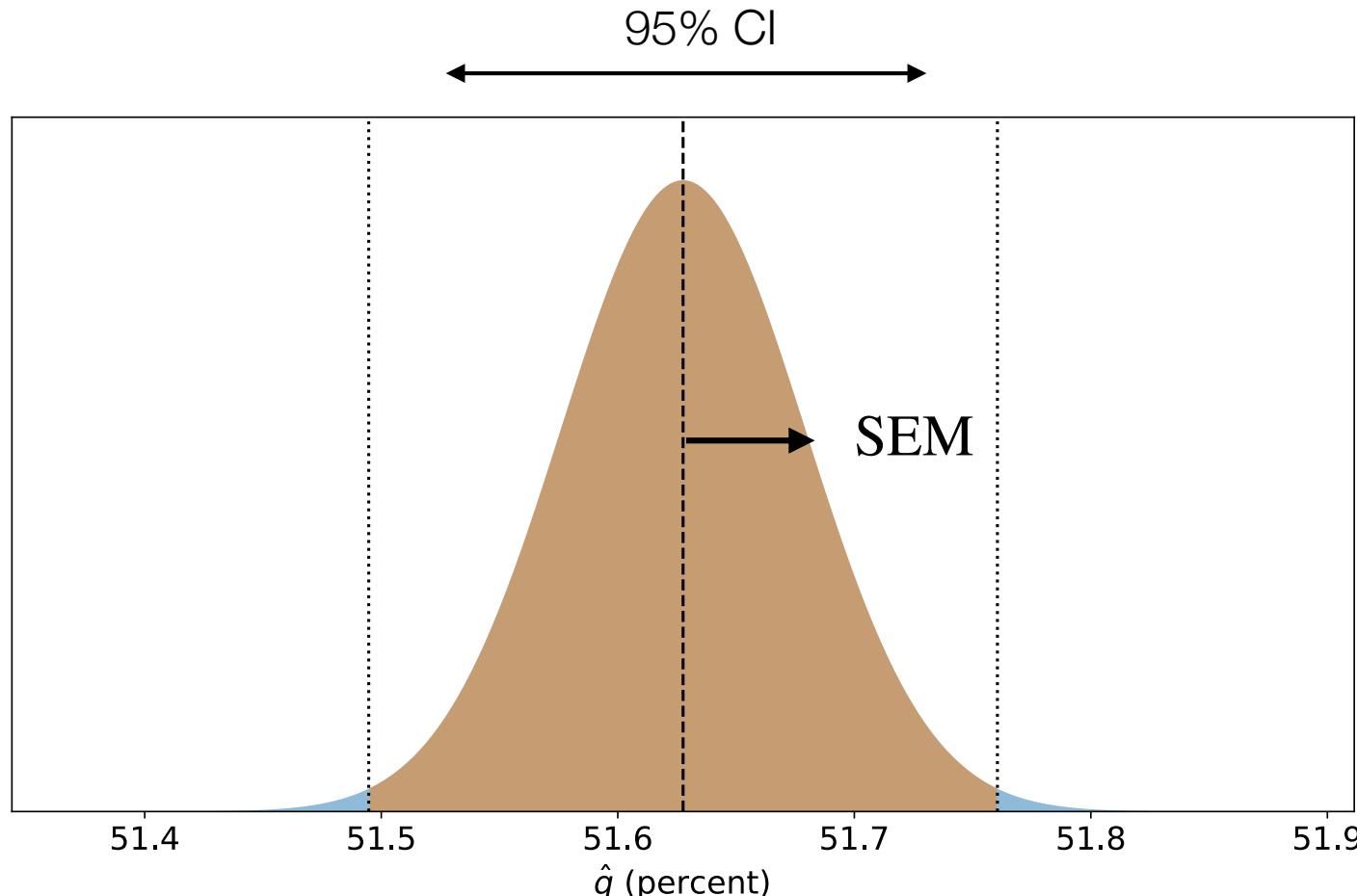
$$\text{SEM} = \sqrt{\hat{q}(1 - \hat{q})/N}$$



The 95% confidence interval, describing plausible values of q , is computed using both \hat{q} and SEM.

The corresponding 95% confidence interval (CI) is

$$[\hat{q} - W, \hat{q} + W] \text{ where } W = 1.96 \times \text{SEM}$$



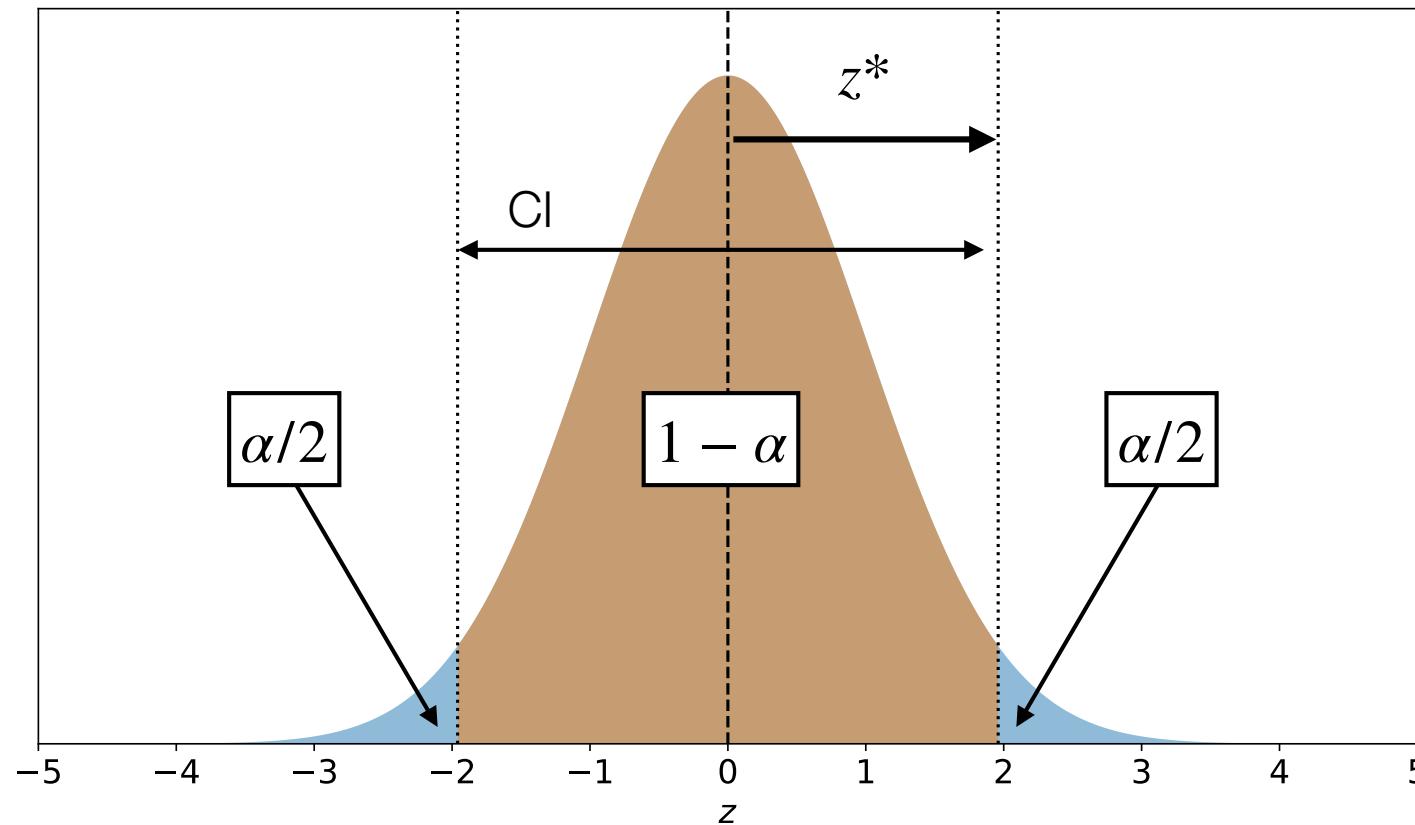
Uncertainty in q is summarized by a z -statistic

The z -statistic is defined by: $z = \frac{q - \hat{q}}{\text{SEM}}$

Because of the central limit theorem, $z \sim \text{Normal}(0, 1)$.

The user chooses a value for α , the probability that q is not within the confidence interval.

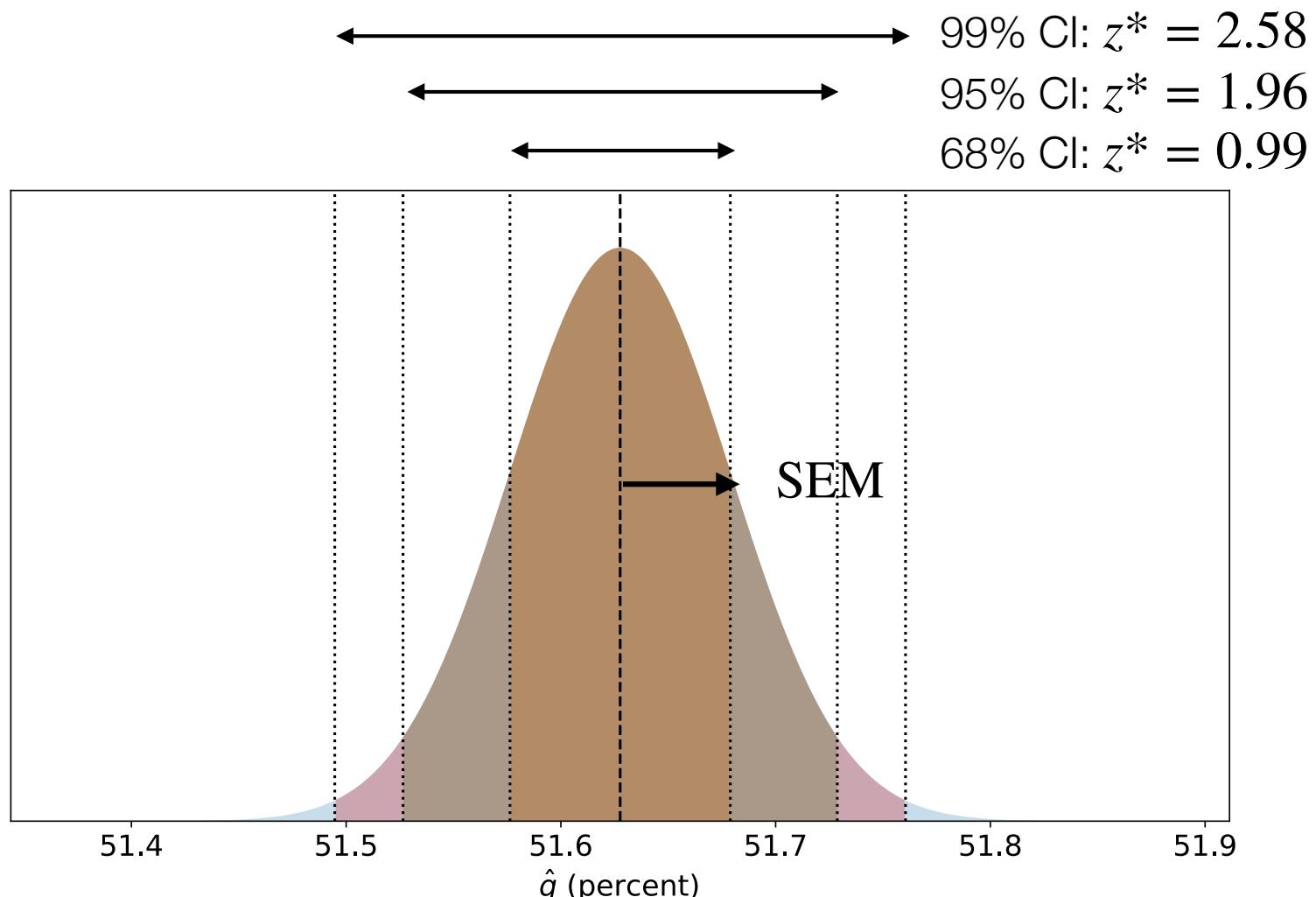
Choosing α fixes the value of z^* . Using $\alpha = 5\%$ gives $z^* = 1.96$.



Confidence intervals of different stringency can be computed using different z-statistic thresholds

Other confidence intervals are given by $[\hat{q} - W, \hat{q} + W]$ where

margin of error: $W = z^* \times \text{SEM}$



Example 2: Healthy Human Body Temperature

Example 2: Human body temperature

Body Temp	Sex	Heart Rate
96.3	2	70
96.7	2	71
96.9	2	74
97.0	2	80
97.1	2	73
97.1	2	75
97.1	2	82
97.2	2	64
97.3	2	69
97.4	2	70

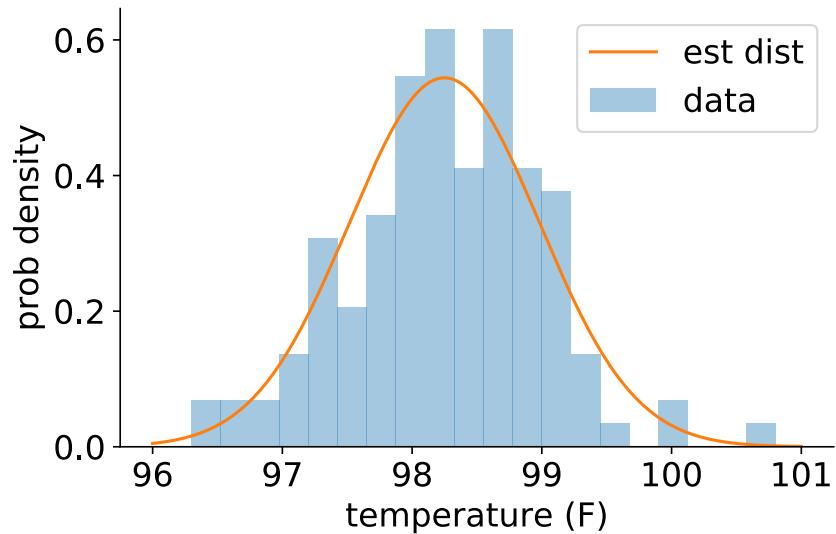
:

Mackowiak PA, Wasserman SS, Levine MM. (1992) A Critical Appraisal of 98.6°F, the Upper Limit of the Normal Body Temperature, and Other Legacies of Carl Reinhold August Wunderlich. *JAMA*. 268(12):1578–1580.

Example 2: Human Body Temperature

We model temperature using a normal distribution

Body Temp
96.3
96.7
96.9
97.0
97.1
97.1
97.1
97.2
97.3
97.4



temperature mean μ

estimate: 98.25 F

95% CI: [98.12 F, 98.38 F]

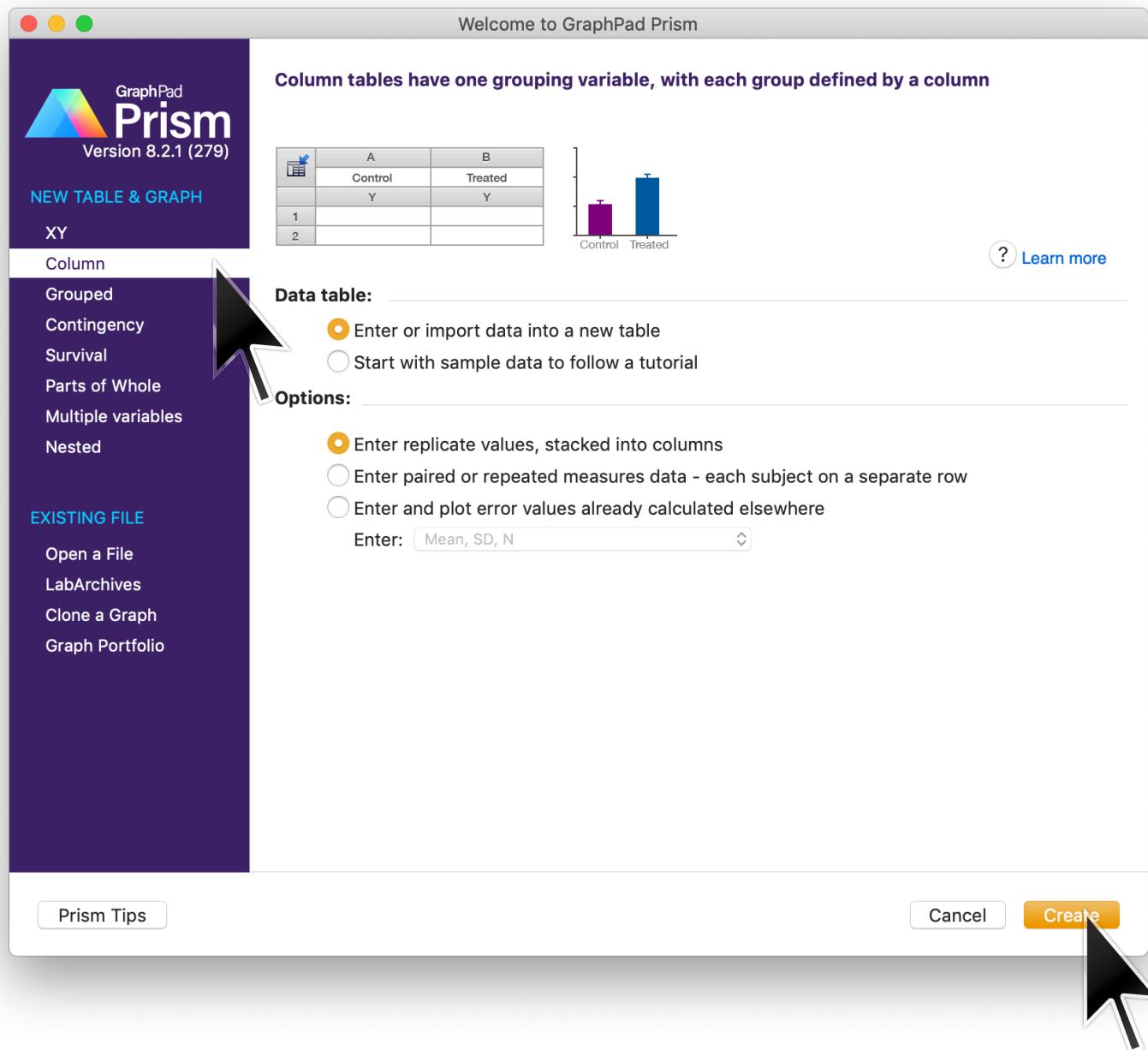
⋮

temperature standard deviation σ

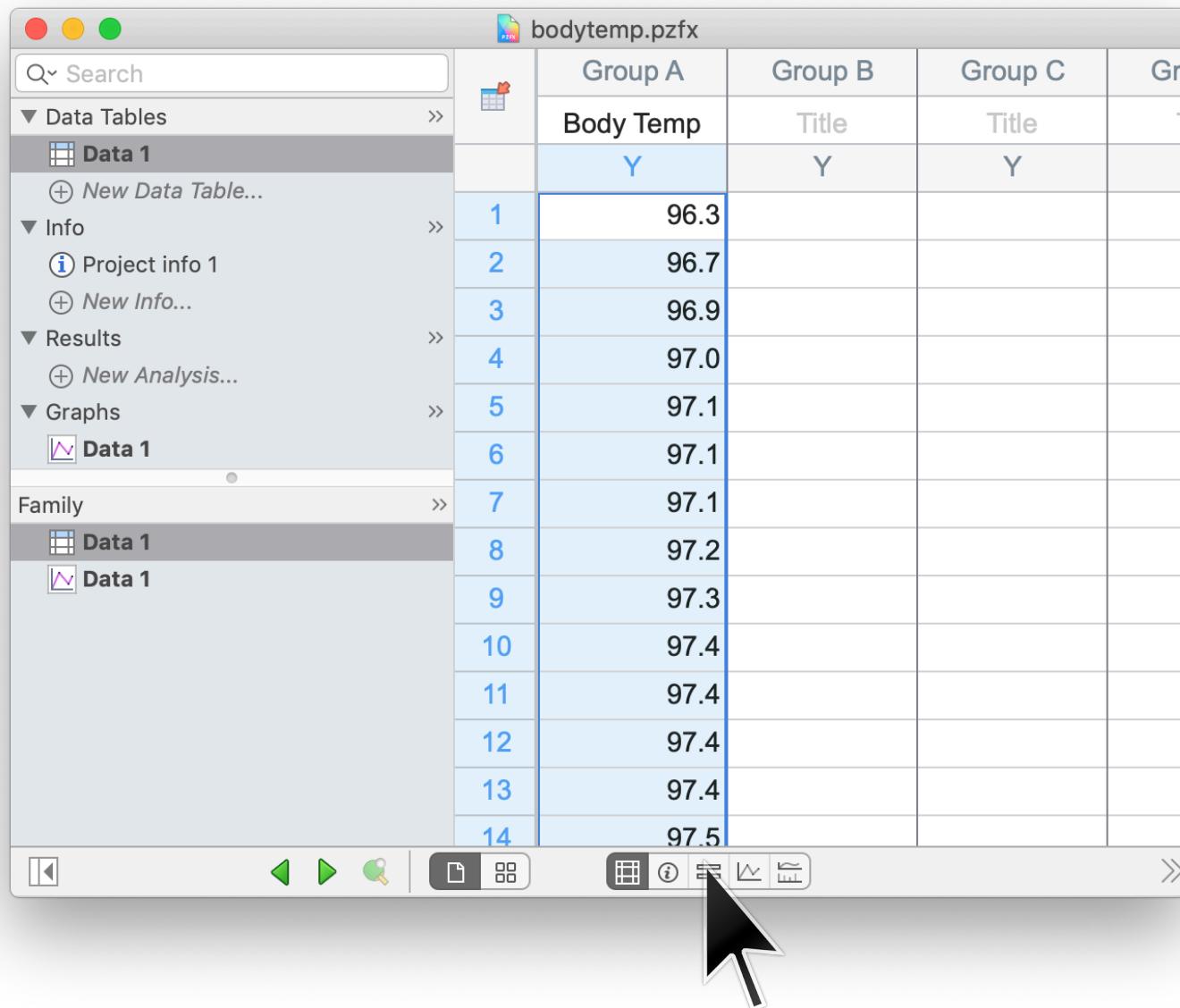
estimate: 0.73 F

95% CI: [0.65 F, 0.83 F]

How to do this in PRISM



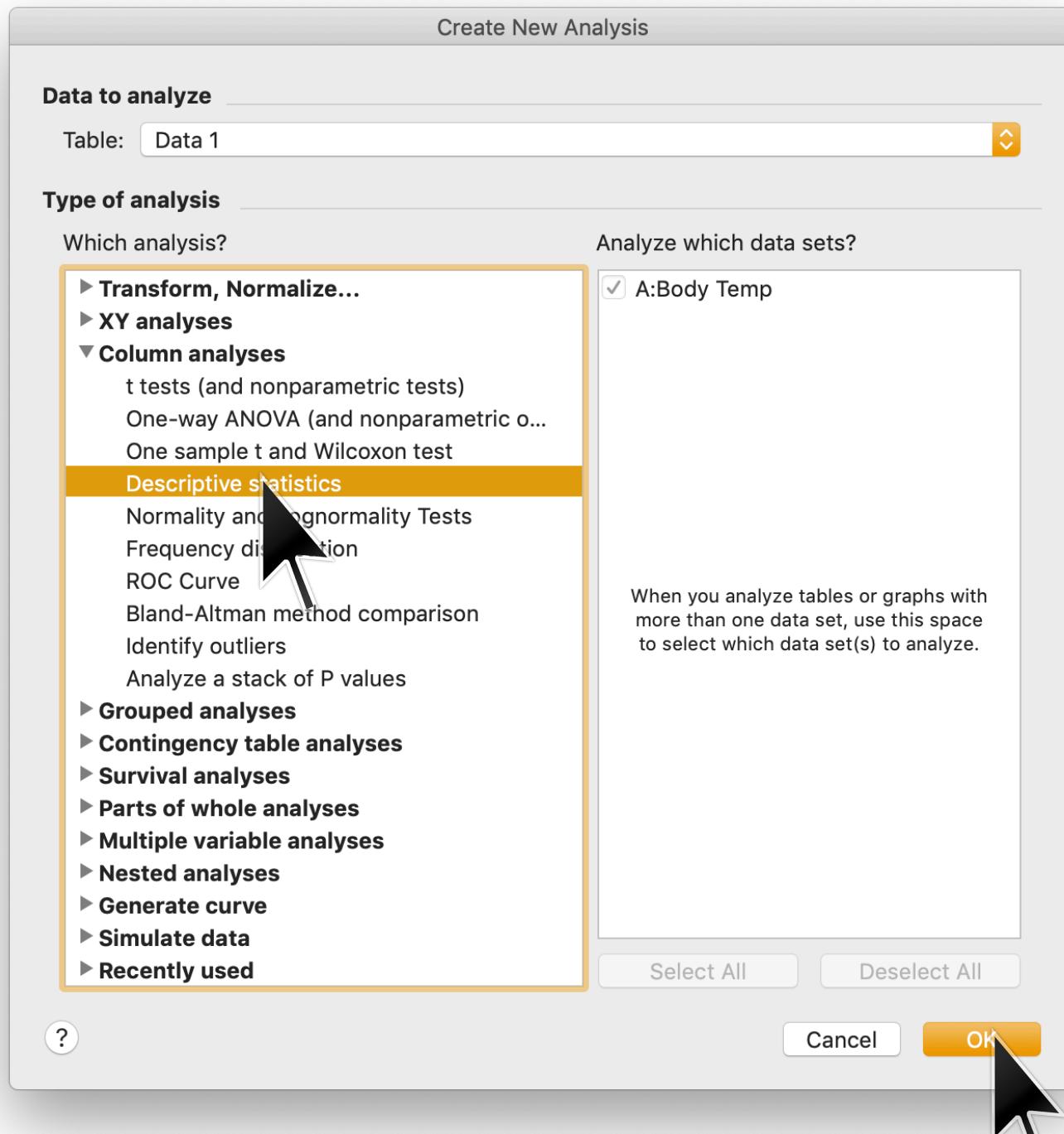
How to do this in PRISM



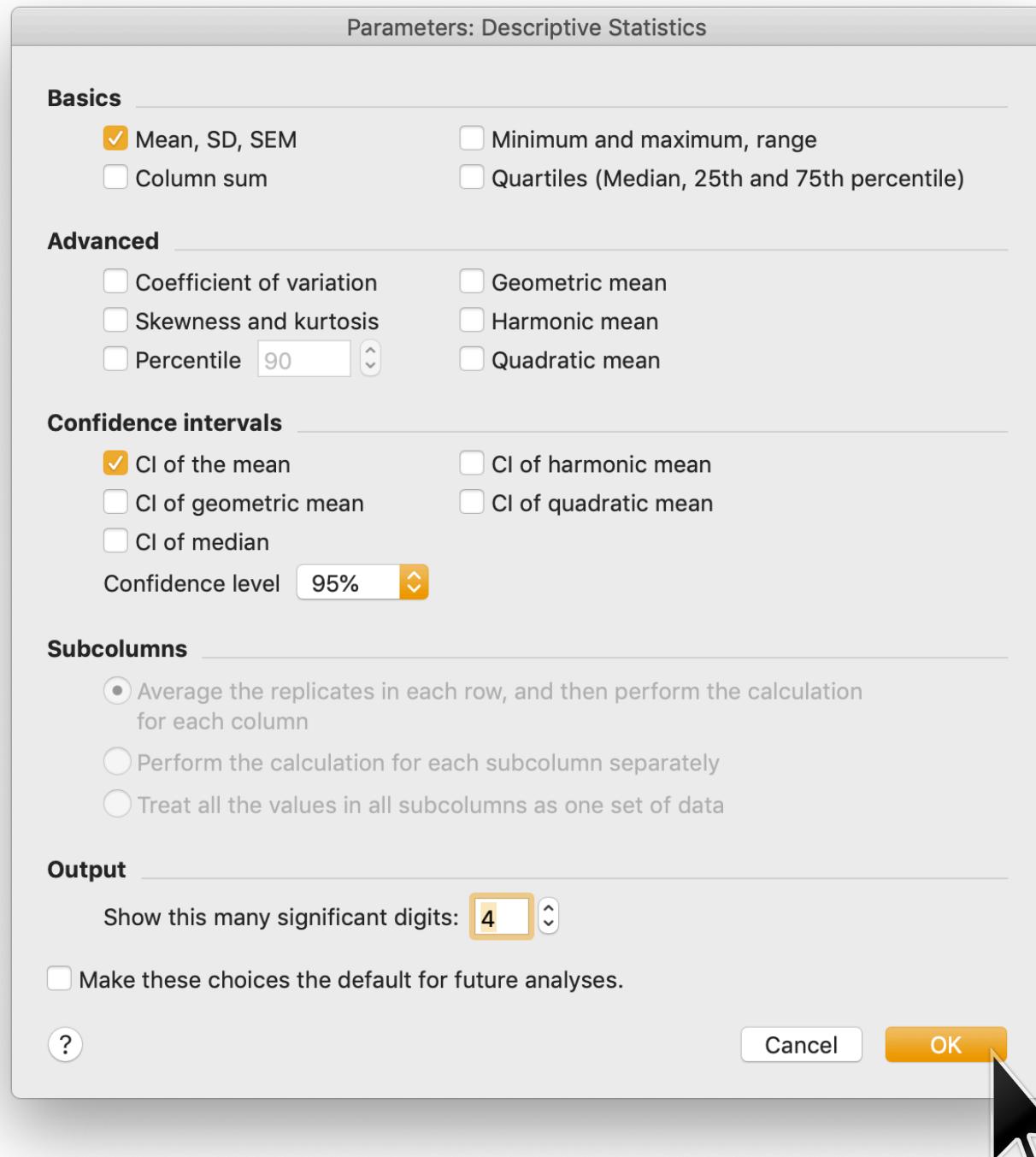
bodytemp.pzfx

	Group A	Group B	Group C	Group D
	Body Temp	Title	Title	Title
	Y	Y	Y	Y
1	96.3			
2	96.7			
3	96.9			
4	97.0			
5	97.1			
6	97.1			
7	97.1			
8	97.2			
9	97.3			
10	97.4			
11	97.4			
12	97.4			
13	97.4			
14	97.5			

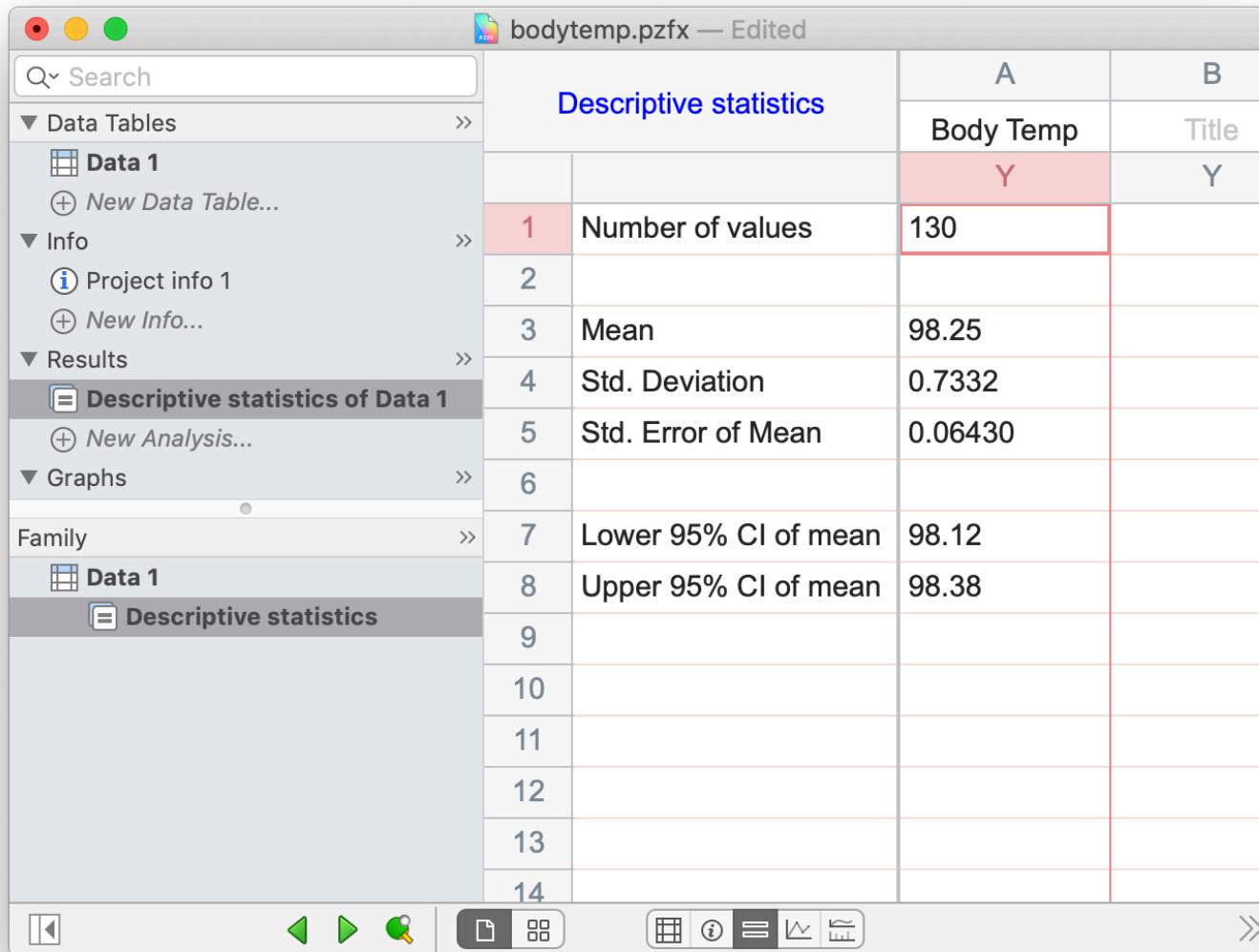
How to do this in PRISM



How to do this in PRISM



How to do this in PRISM



The screenshot shows the PRISM software interface with the project file "bodytemp.pzfx — Edited". The left sidebar contains a search bar and sections for Data Tables, Info, Results, and Graphs. The "Results" section is expanded, showing "Descriptive statistics of Data 1" and "New Analysis...". The "Graphs" section is collapsed. The main area displays a table titled "Descriptive statistics" for "Data 1". The table has two columns: "A" and "B". The "A" column contains numerical values and labels (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14). The "B" column contains descriptive statistics: "Body Temp" (Title: Y, Value: 130), "Number of values" (Value: 130), "Mean" (Value: 98.25), "Std. Deviation" (Value: 0.7332), "Std. Error of Mean" (Value: 0.06430), "Lower 95% CI of mean" (Value: 98.12), and "Upper 95% CI of mean" (Value: 98.38). The table is styled with alternating row colors and red borders for certain cells.

Descriptive statistics	
A	B
	Body Temp
1	Number of values
2	
3	Mean
4	Std. Deviation
5	Std. Error of Mean
6	
7	Lower 95% CI of mean
8	Upper 95% CI of mean
9	
10	
11	
12	
13	
14	

We assume the temperature of a healthy person is drawn from a normal distribution

data

$$x_1, x_2, \dots, x_N$$

x_i : temperature of individual i in Fahrenheit

model

$$x \sim \text{Normal}(\mu, \sigma^2)$$

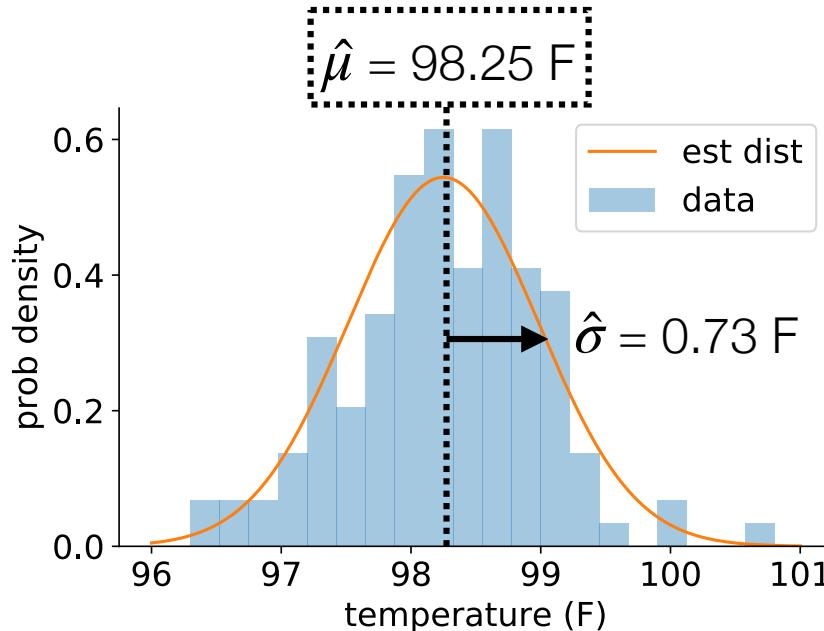
μ : average body temperature

σ : standard deviation of temperatures

goals

1. Compute best estimates for both μ, σ
2. Compute confidence intervals for both μ, σ

We want to infer two parameters from our data



Here there are two parameters that need to be estimated, μ and σ

This is unlike with the binomial distribution, where there was only one parameter q .

The lingering uncertainty in μ is described by a t-distribution

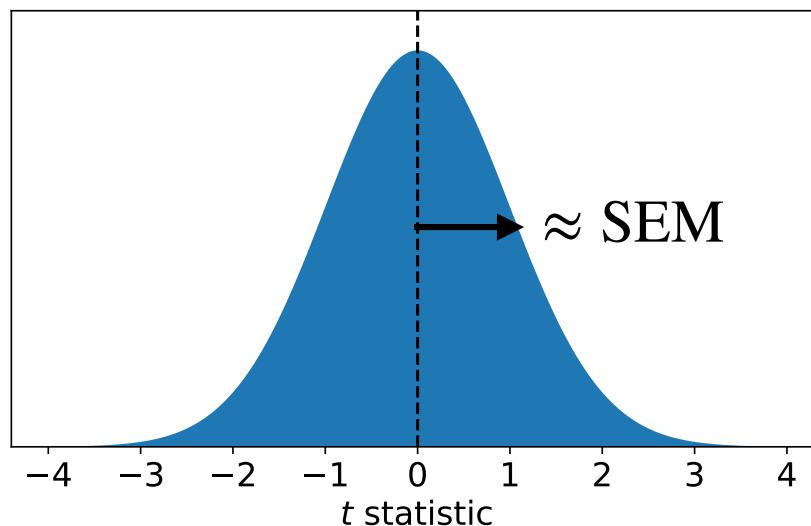
The standard error of the mean (SEM) is given by

$$\text{SEM} = \frac{\hat{\sigma}}{\sqrt{N}}$$

A t-statistic is then used to indicate how strongly μ deviates from $\hat{\mu}$:

$$t = \frac{\mu - \hat{\mu}}{\text{SEM}}$$

The t-statistic follows a t-distribution
(almost a normal distribution, but not quite)

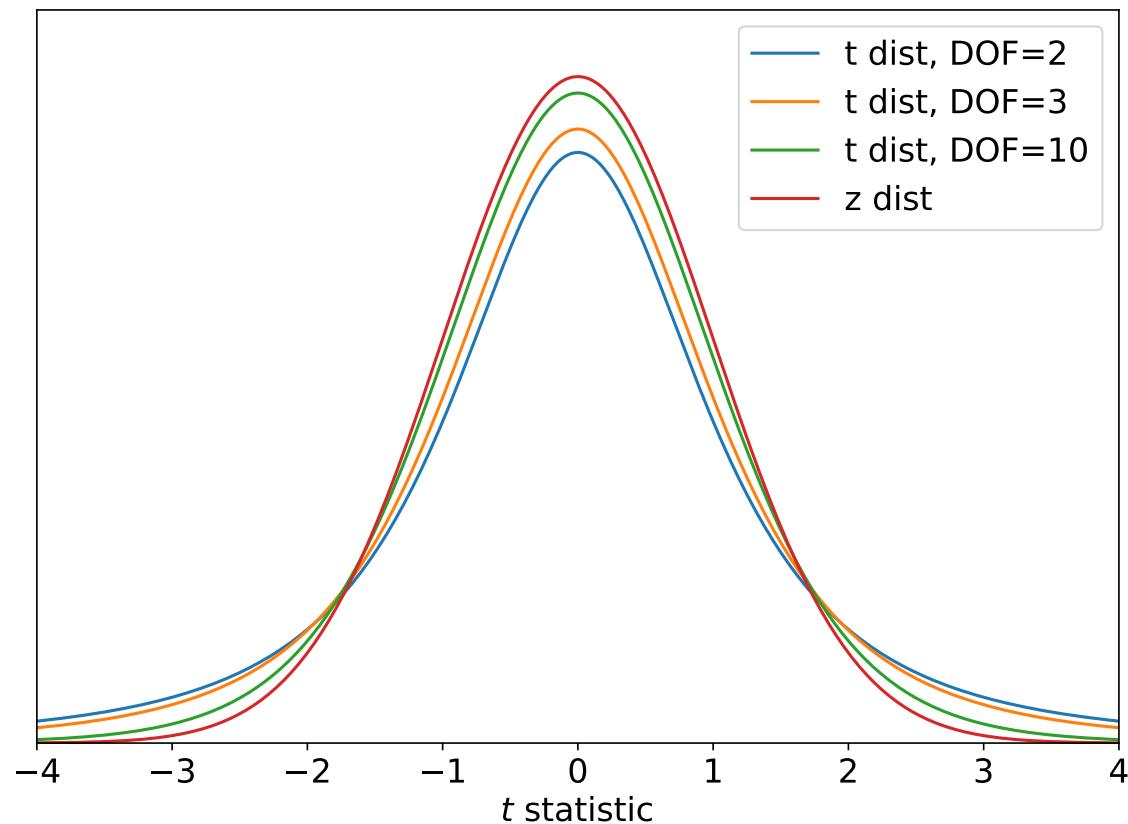


The shape of the t-distribution is affected by the number of degrees of freedom (DOF)

In this case, we use a t-distribution with DOF given by

$$\text{DOF} = N - 1$$

This is almost indistinguishable from a normal (z) distribution when $\text{DOF} \gtrsim 10$.

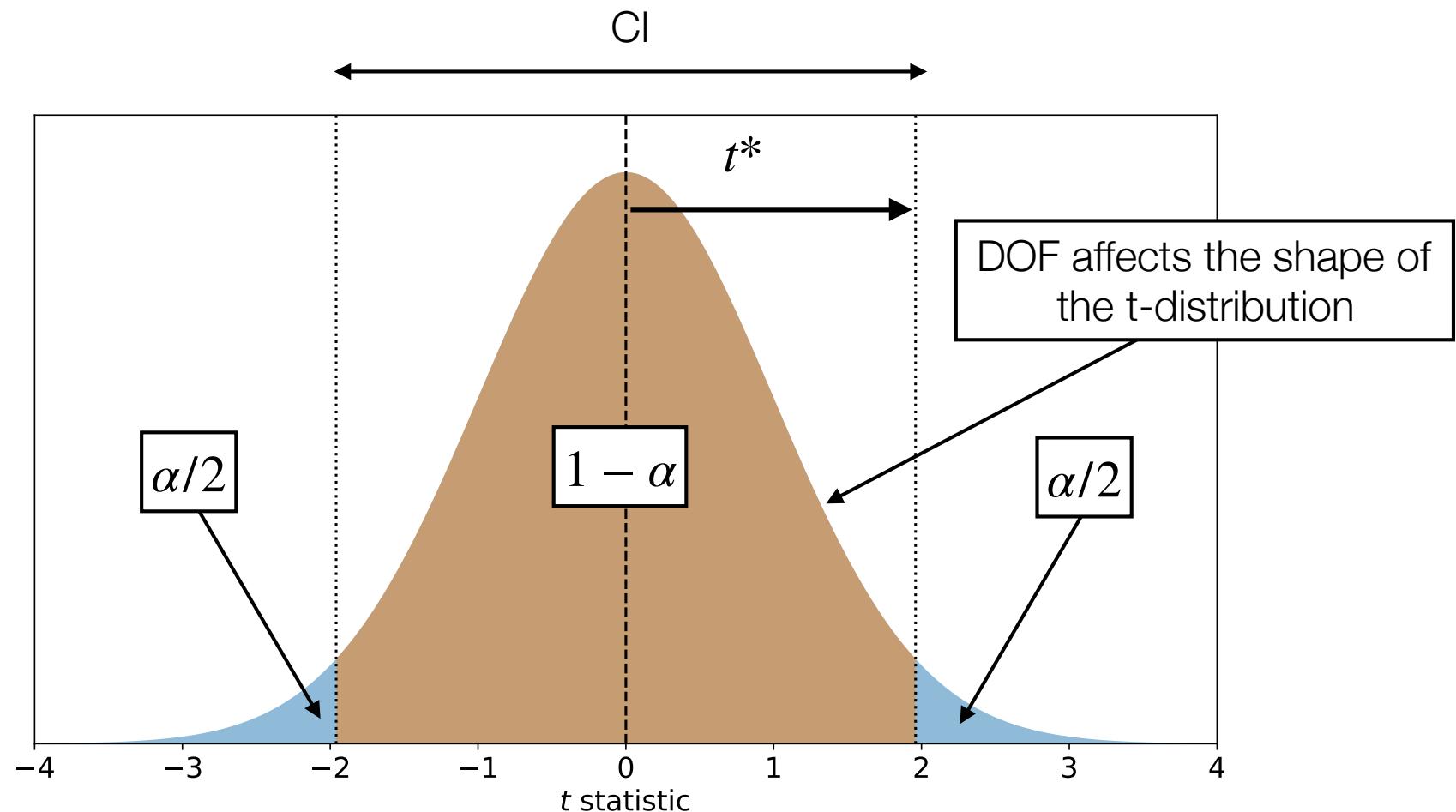


The t-distribution is used to compute a t-statistic cutoff, which determines the confidence interval

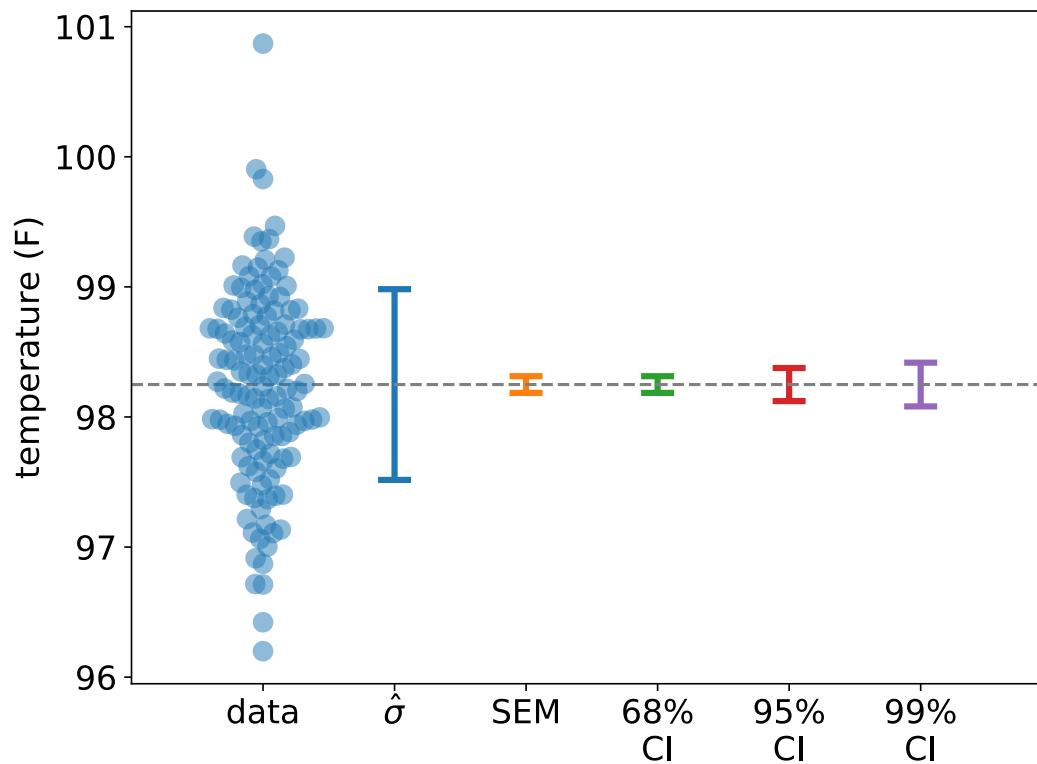
The t-statistic cutoff, t^* , is determined by both α and the DOF.

$$\text{margin of error: } W = t^* \cdot \text{SEM}$$

$$\text{confidence interval: } \hat{\mu} \pm W$$



Confidence intervals (CIs) and standard errors of the mean (SEMs) quantify how uncertain a parameter



SEMs and CIs of the mean quantify the uncertainty in μ ,
not the width of the sampling distribution ($\hat{\sigma}$).

SEMs and CIs decrease in size as the amount of data increases.

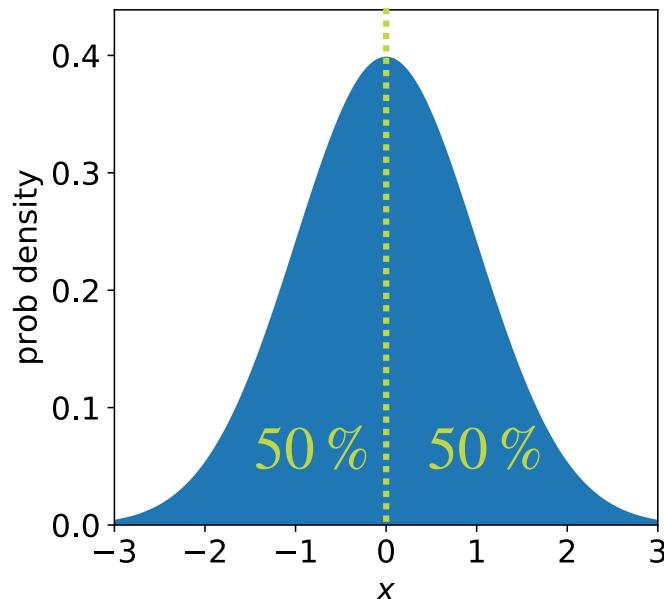
CIs increase in size if the required confidence level increases (i.e., α decreases)

The median is the standard nonparametric estimate of a distribution's center

For data: sort the data $x_1, x_2, x_3, \dots, x_N$ in ascending order. The median is then defined as:

$$\text{median} = q_{50} = \begin{cases} x_{\frac{N+1}{2}} & \text{if } N \text{ odd} \\ \frac{1}{2} \left(x_{\frac{N}{2}} + x_{\frac{N+2}{2}} \right) & \text{if } N \text{ even} \end{cases}$$

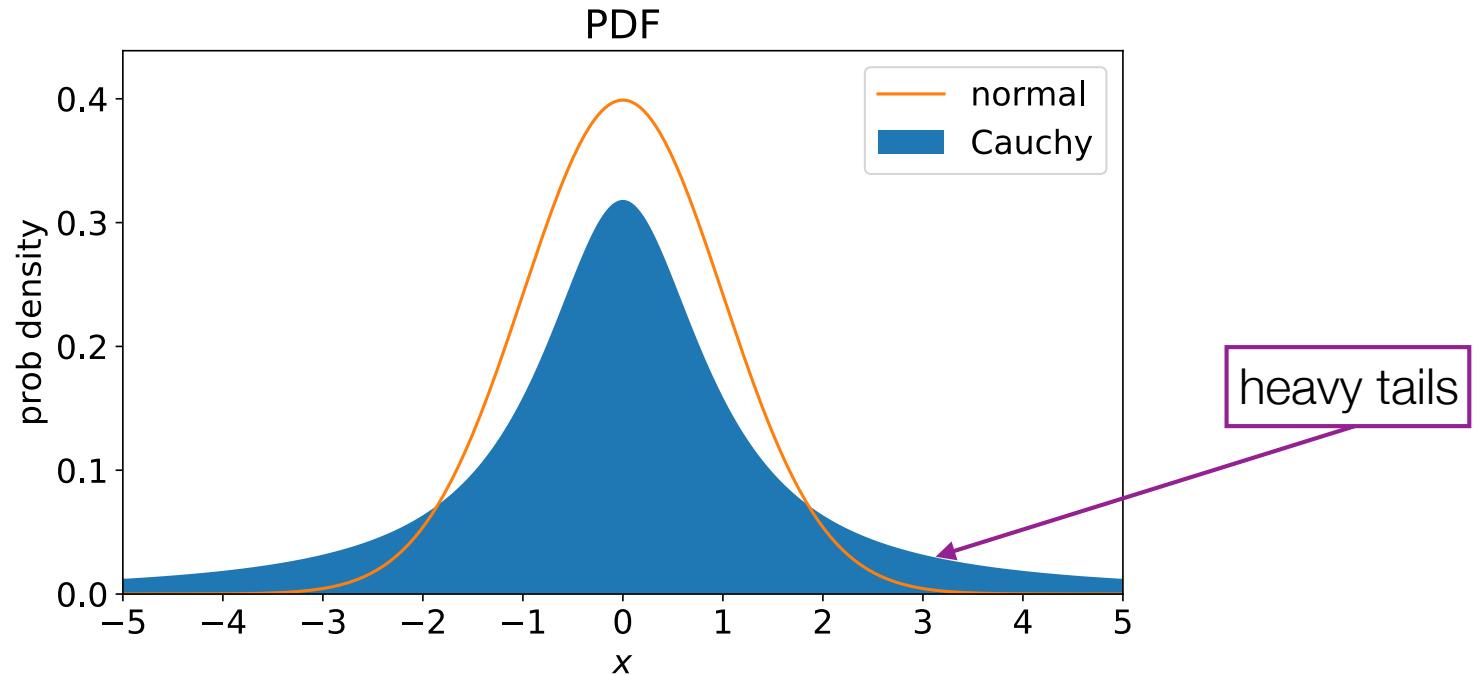
For a distribution: the median is the value of x that separates half the distribution's mass from the other.



The median of a symmetric distribution is equal to its mean

The median is less sensitive to outliers than the mean

Cauchy distribution



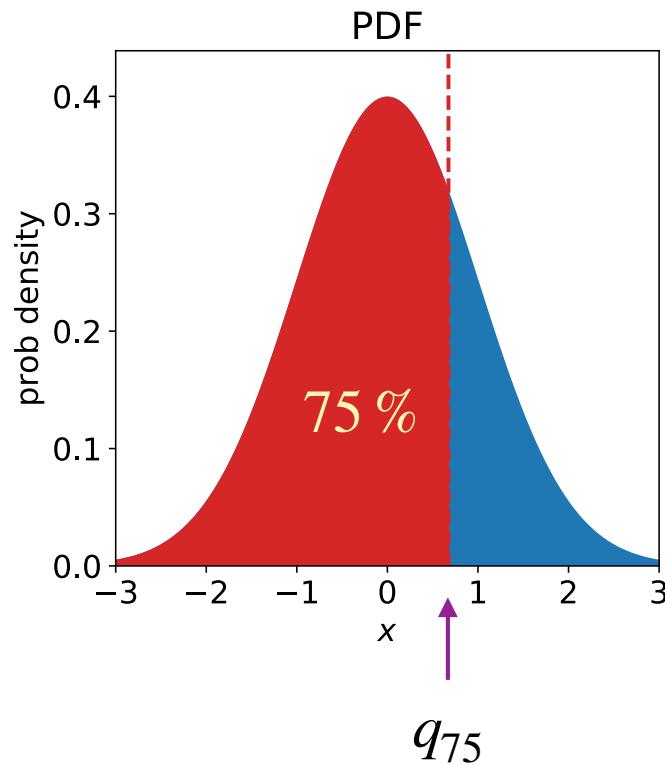
The standard estimate of the mean $\hat{\mu}$ will not converge as N becomes large!

The median q_{50} does converge, just as quickly as for any distribution.

Quantiles of a distribution

More generally, the quantile q_K of a distribution is the value of x that bounds $K\%$ of the distribution's mass.

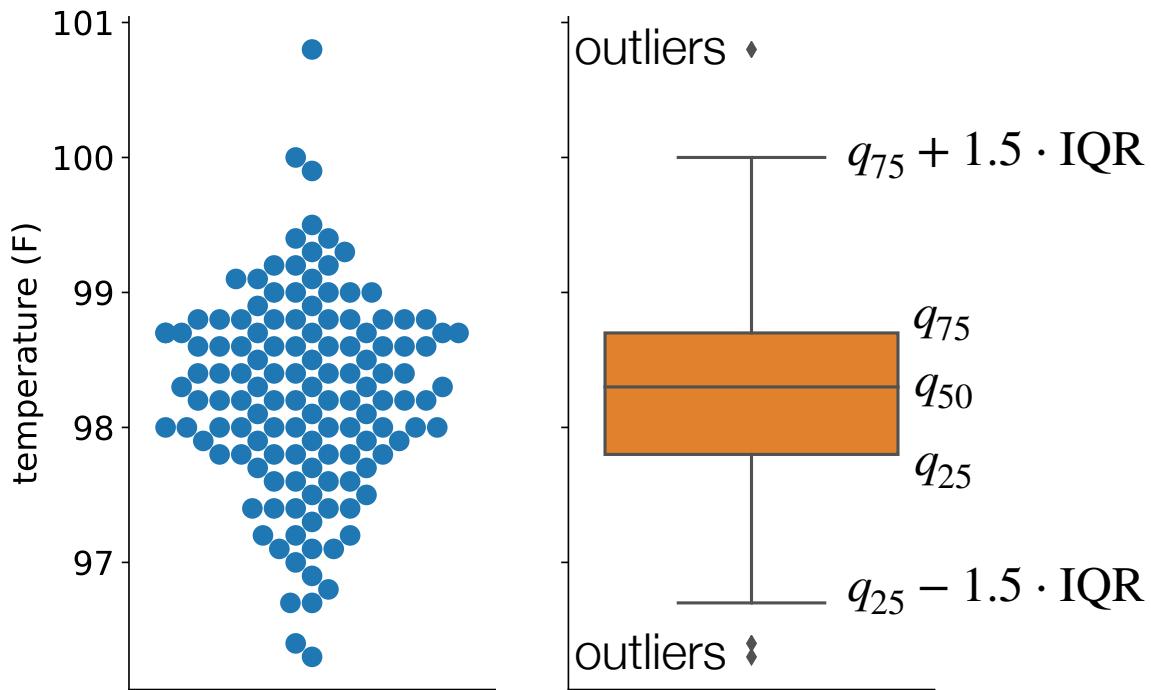
E.g., the median in the quantile q_{50}



Box and whisker plots indicate quantiles

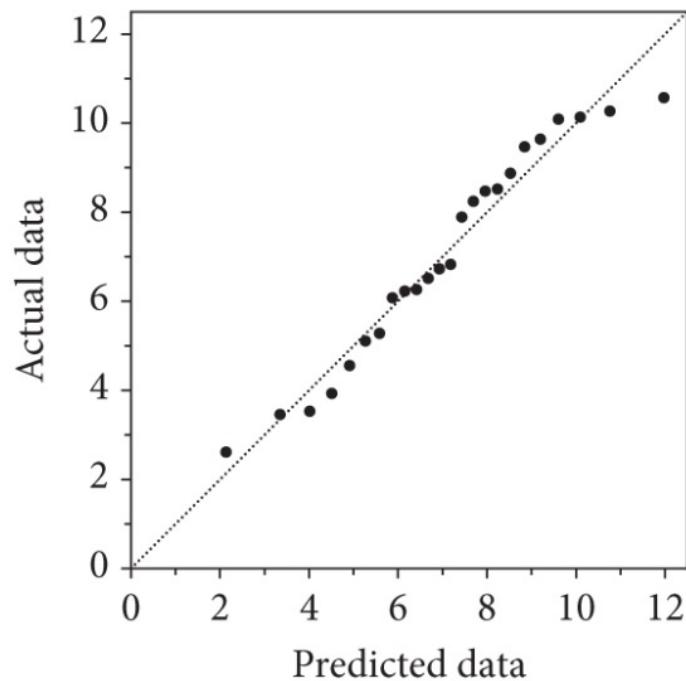
Interquartile range is defined by

$$\text{IQR} = q_{75} - q_{25}$$

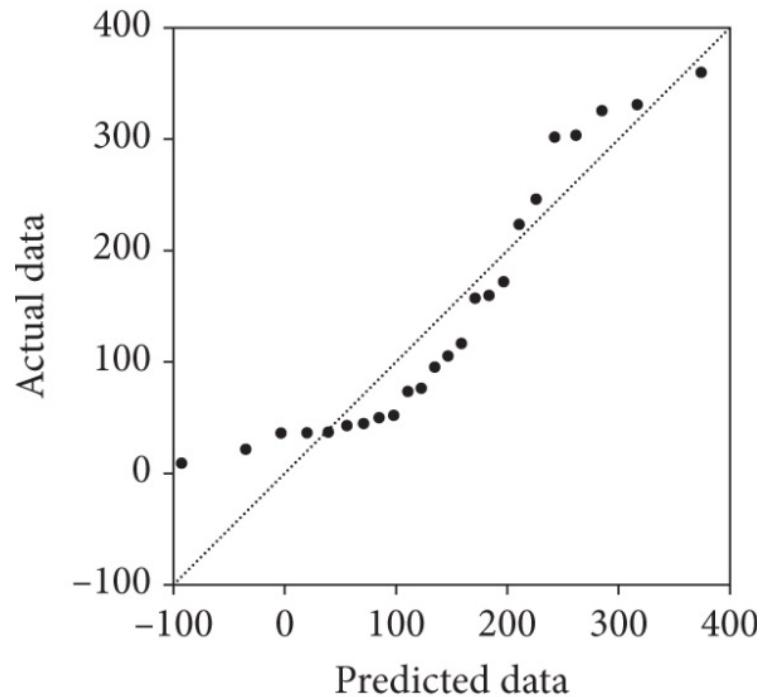


QQ plots are used to visually test whether data follows an expected distribution.

ok agreement



systematic deviation



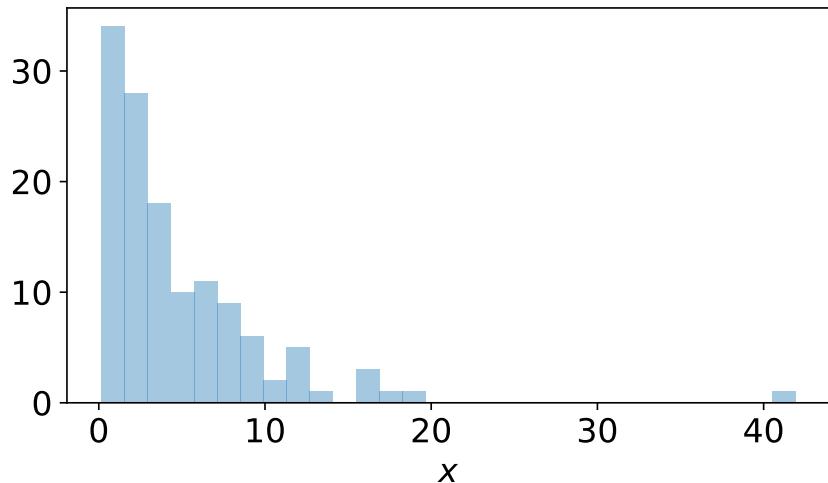
y axis: sorted data values x_1, x_2, \dots, N .

x axis: corresponding quantiles q_X of the inferred distribution, using the percentile values X_1, X_2, \dots, X_N computed for each data point.

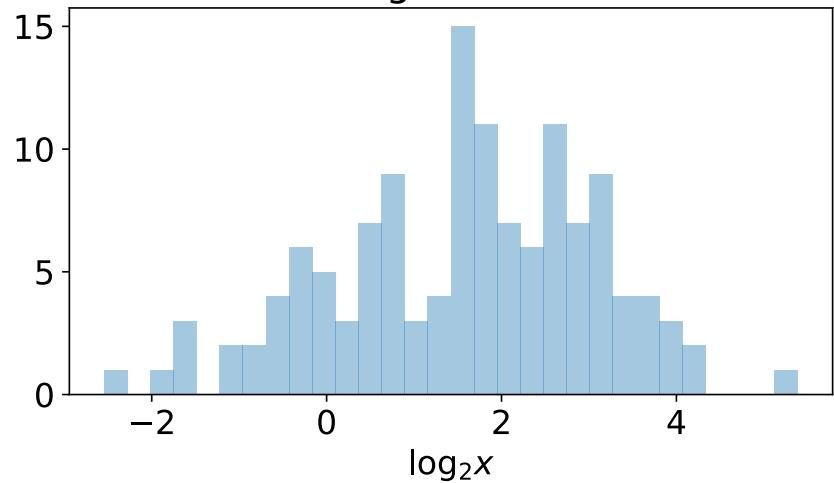
The analysis of a QQ plot is done by eye and making a judgement call.

QQ plot example: simulated lognormal data

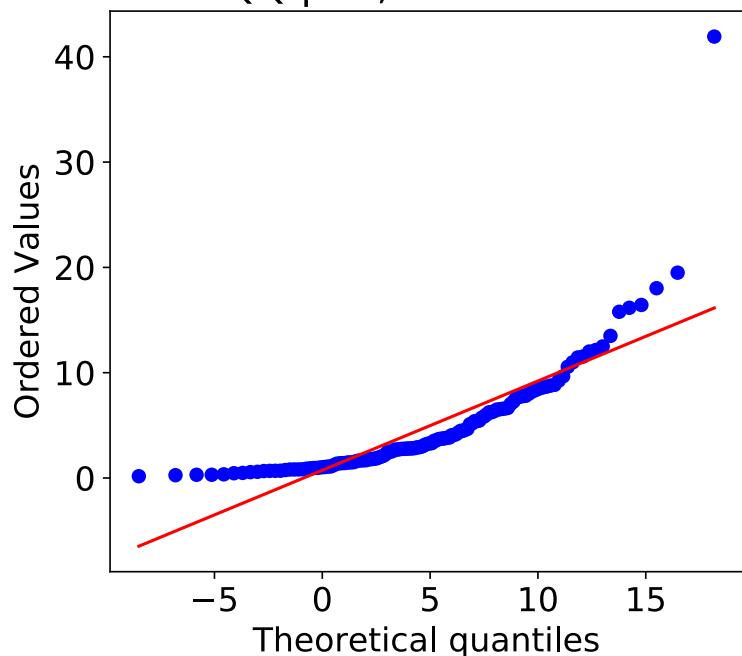
linear scale



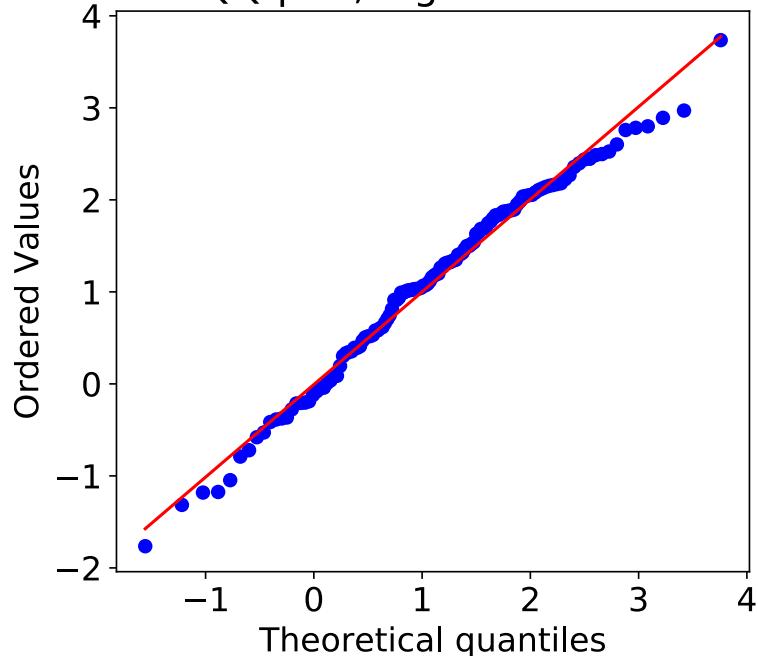
log2 scale



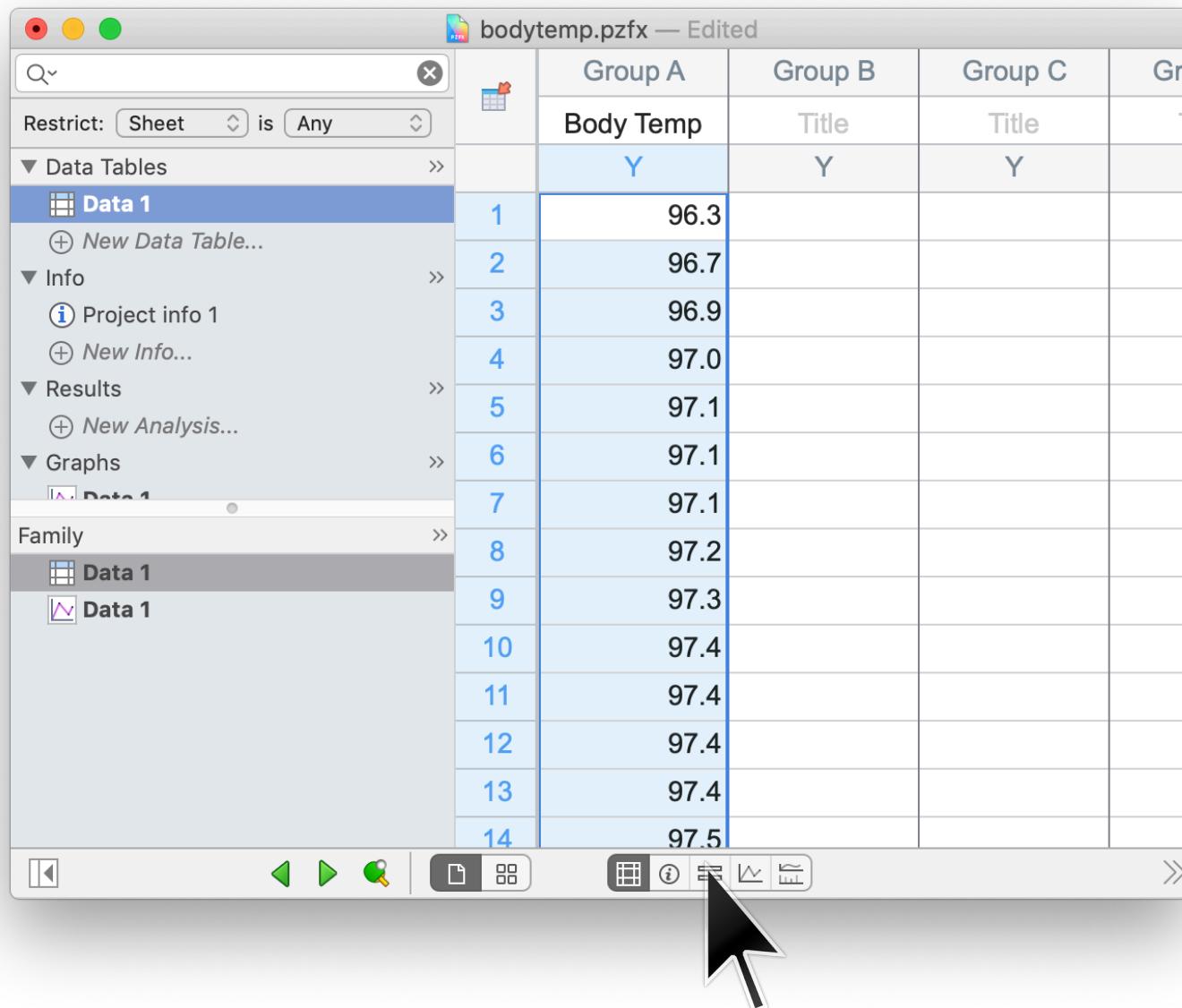
QQ plot, normal dist



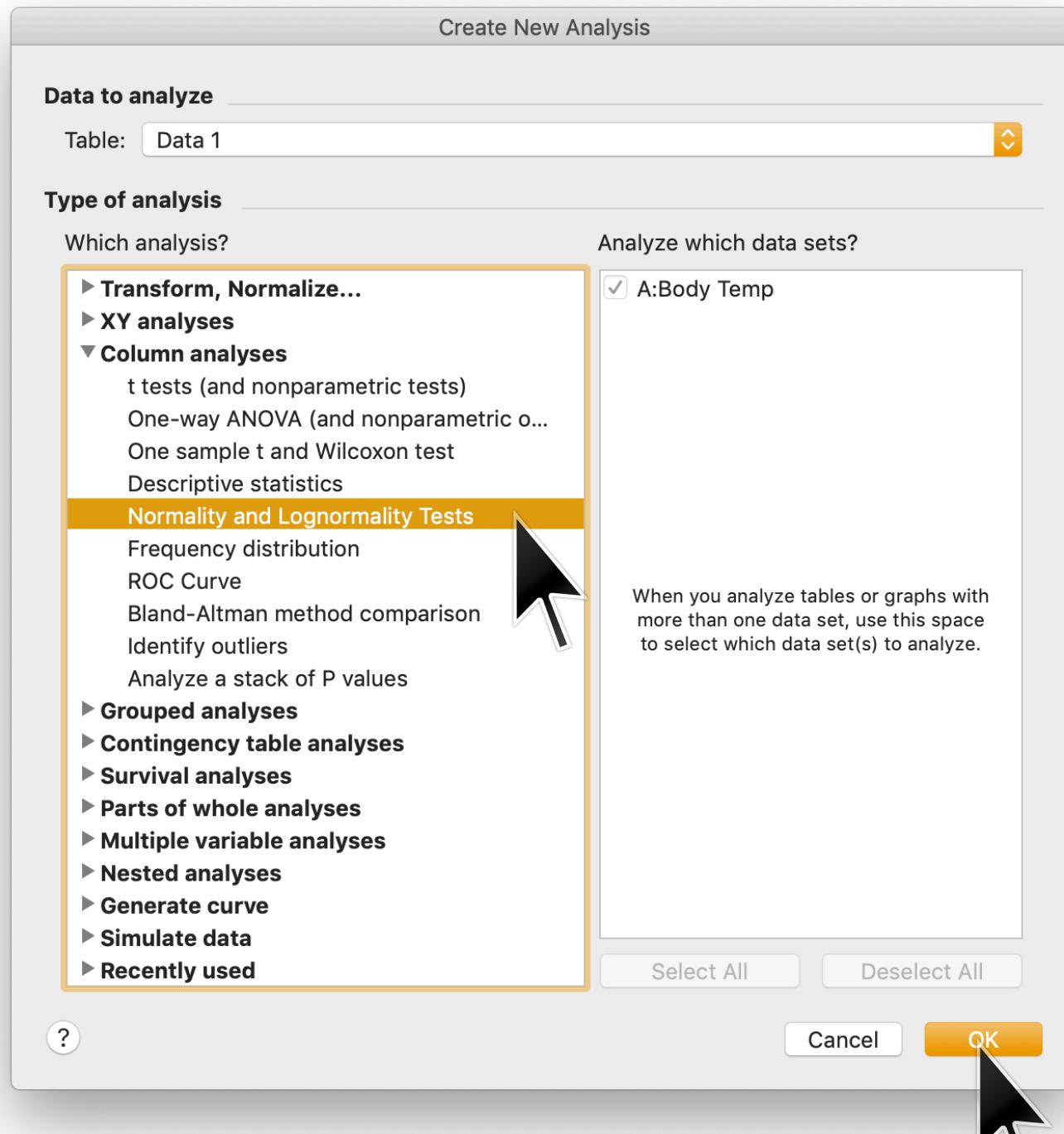
QQ plot, lognormal dist



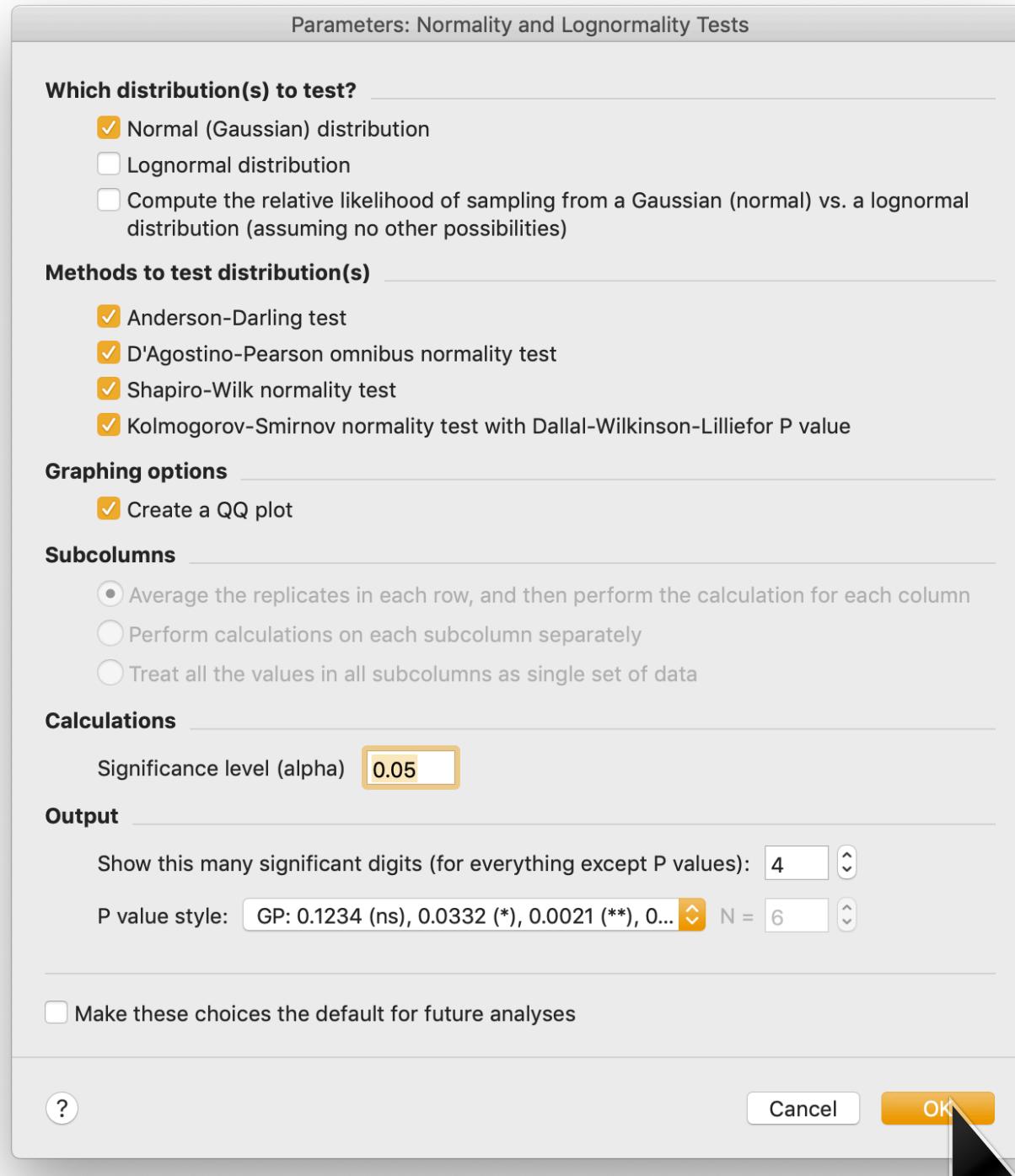
How to do this in Prism



How to do this in Prism



How to do this in Prism



How to do this in Prism

bodytemp.pzfx — Edited

Restrict: Sheet is Any

Data Tables

- Data 1
- New Data Table...

Info

- Project info 1
- New Info...

Results

Normality and Lognormality Tests

- New Analysis...

Graphs

- Data 1
- Normal QQ plot: Normality and Lognormality Tests
- New Graph...

Layouts

Family

- Data 1
- Normality and Lognormality Tests
- Normal QQ plot: Normality and Lognormality Tests

Tabular results

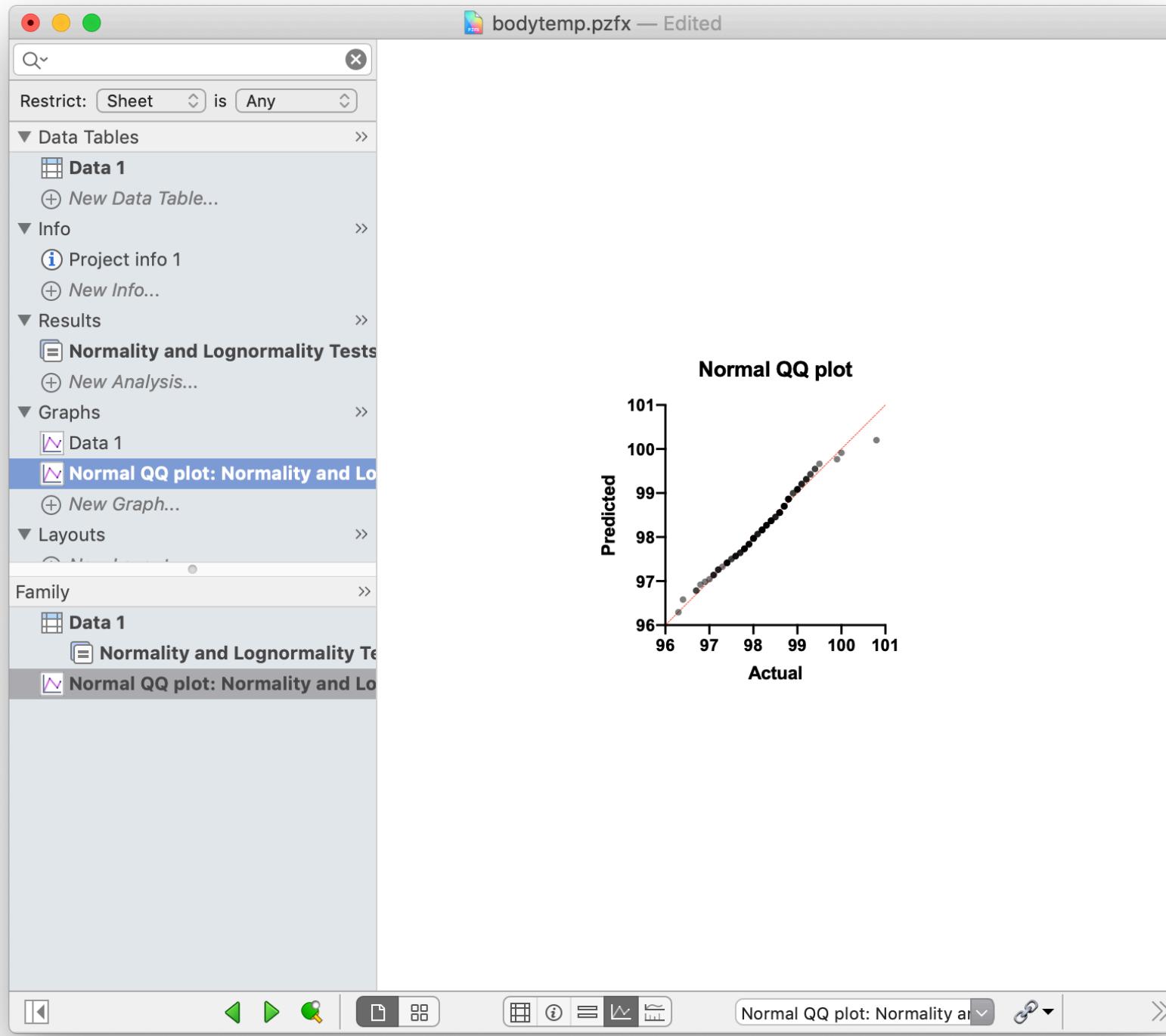
Normality and Lognormality Tests

Tabular results

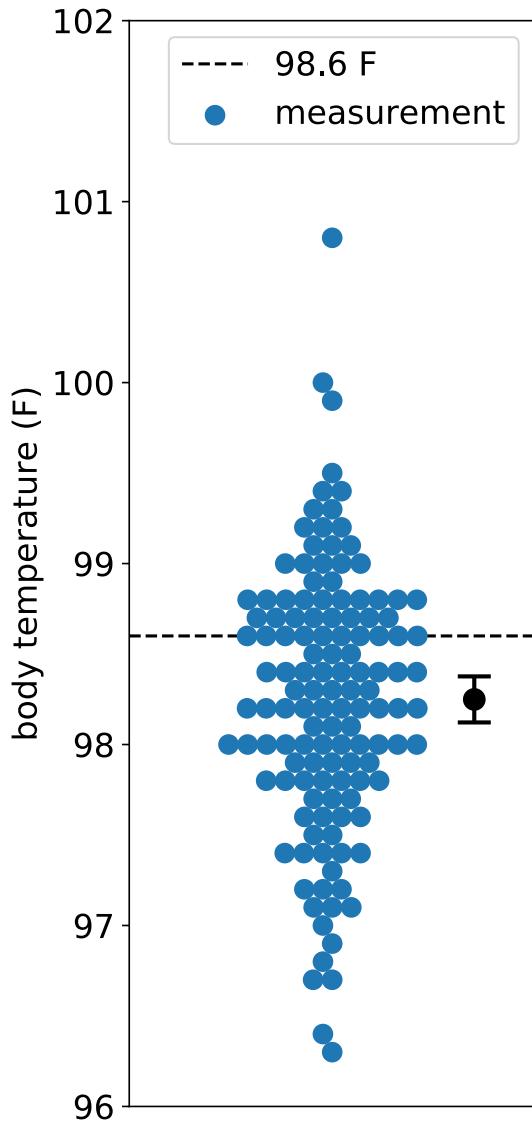
	A	B	C	D
	Body Temp	Title	Title	Title
1	Test for normal distribution	Y	Y	Y
2	Anderson-Darling test			
3	A2*	0.5201		
4	P value	0.1829		
5	Passed normality test (alpha=0.05)?	Yes		
6	P value summary	ns		
7				
8	D'Agostino & Pearson test			
9	K2	2.704		
10	P value	0.2587		
11	Passed normality test (alpha=0.05)?	Yes		
12	P value summary	ns		
13				
14	Shapiro-Wilk test			
15	W	0.9866		
16	P value	0.2332		
17	Passed normality test (alpha=0.05)?	Yes		
18	P value summary	ns		
19				
20	Kolmogorov-Smirnov test			
21	KS distance	0.06473		
22	P value	>0.1000		
23	Passed normality test (alpha=0.05)?	Yes		
24	P value summary	ns		
25				
26	Number of values	130		
27				

Normality and Lognormality

How to do this in PRISM



Student's t test (single sample)



Null Hypothesis:

a population is normally distributed with
a known mean value of μ_{null}

Data:

measurements: x_1, x_2, \dots, x_N

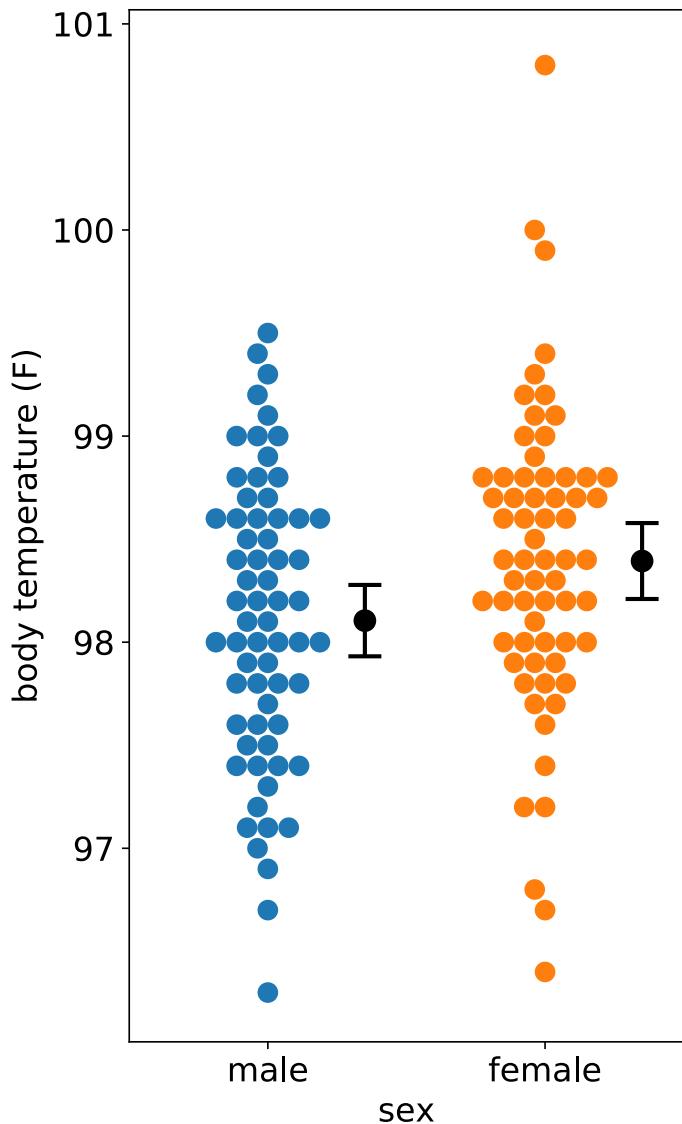
Test statistic:

$$t = \frac{\hat{\mu} - \mu_{\text{null}}}{\text{SEM}}$$

Null distribution:

t distribution with $\text{DOF} = N - 1$.

Student's t test (two sample, equal variance)



Null Hypothesis:

two populations have the same mean

Data:

x_1, x_2, \dots, x_m and y_1, y_2, \dots, y_n

Assumptions:

the two populations follow normal distributions and have equal variances

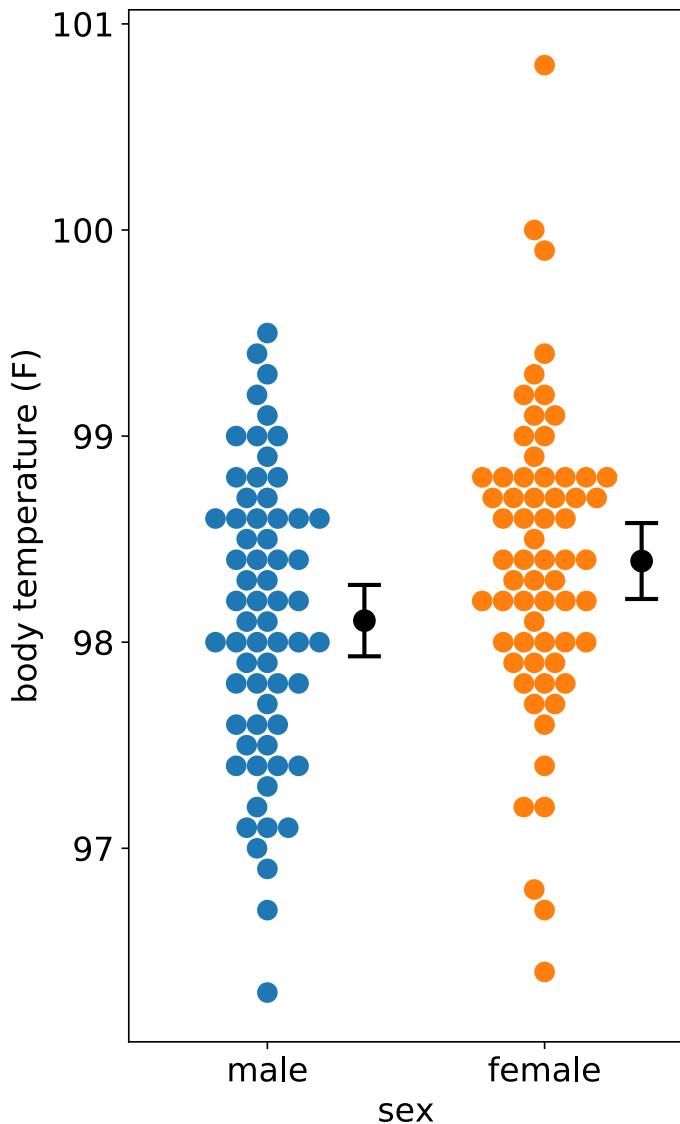
Test statistic:

$$t = \frac{\hat{\mu}_x - \hat{\mu}_y}{\hat{\sigma} \sqrt{\frac{1}{m} + \frac{1}{n}}}, \quad \hat{\sigma} = \sqrt{\frac{(m-1)\hat{\sigma}_x^2 + (n-1)\hat{\sigma}_y^2}{m+n-2}}$$

Null distribution:

t distribution with $DOF = m + n - 2$.

Welch's t test



Null Hypothesis:

two populations have the same mean but
not necessarily the same standard deviation

Data:

x_1, x_2, \dots, x_m and y_1, y_2, \dots, y_n

Advantage:

Fewer assumptions than standard unpaired t test

Disadvantage:

Less power than standard unpaired t tests

Test statistic:

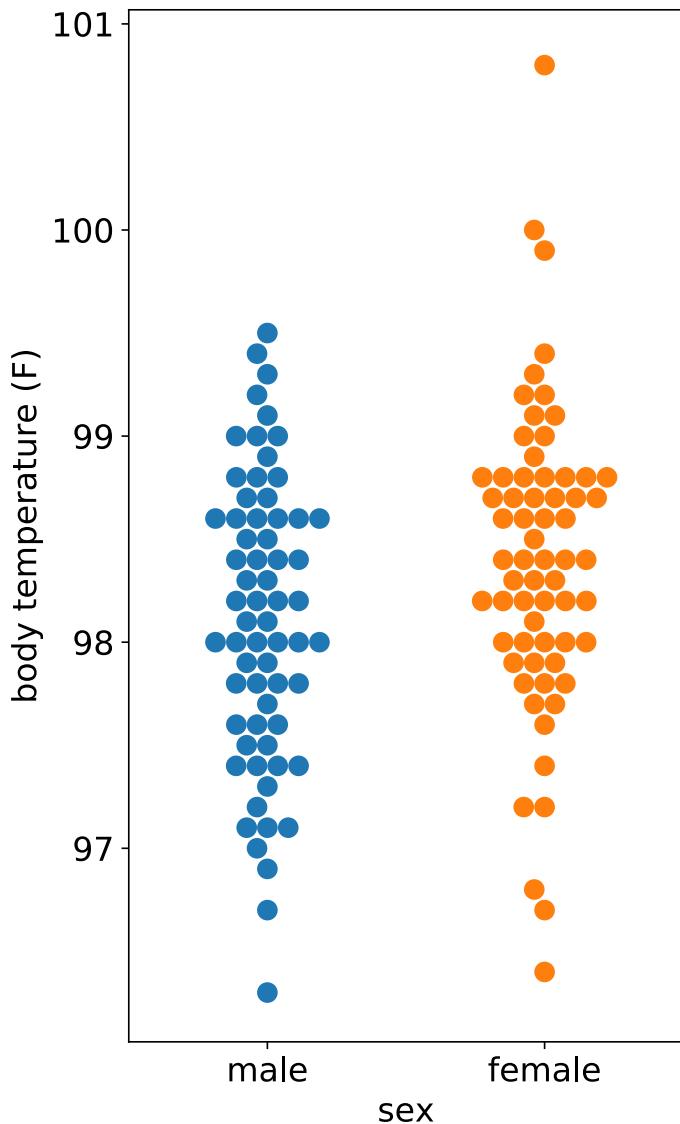
$$t = \frac{\hat{\mu}_x - \hat{\mu}_y}{\sqrt{\frac{\hat{\sigma}_x^2}{m} + \frac{\hat{\sigma}_y^2}{n}}}$$

Null distribution:

Student's t distribution with

$$\text{DOF} = \frac{\left(\frac{\hat{\sigma}_x^2}{m} + \frac{\hat{\sigma}_y^2}{n} \right)^2}{\frac{(\hat{\sigma}_x^2/m)^2}{m-1} + \frac{(\hat{\sigma}_y^2/n)^2}{n-1}}$$

Mann Whitney U test



Null Hypothesis:

If x is sampled from population 1 and y is sampled from population 2,

$$p(x > y) = p(x < y)$$

Data:

$$x_1, x_2, \dots, x_m \text{ and } y_1, y_2, \dots, y_n$$

Advantage:

No assumptions about the mathematical form of $p(x)$ and $p(y)$.

Disadvantage:

Somewhat less powerful than Student's t test

Test statistic:

$$U \text{ (based on rank-order of } xs \text{ and } ys)$$

temp_by_sex.pzfx

	Group A	Group B	Group C	Group D
	male	female	Title	Title
1	96.3	96.4		
2	96.7	96.7		
3	96.9	96.8		
4	97.0	97.2		
5	97.1	97.2		
6	97.1	97.4		
7	97.1	97.6		
8	97.2	97.7		
9	97.3	97.7		
10	97.4	97.8		
11	97.4	97.8		
12	97.4	97.8		
13	97.4	97.9		
14	97.5	97.9		

Create New Analysis

Data to analyze

Table: Data 1

Type of analysis

Which analysis?

- ▶ **Transform, Normalize...**
- ▶ **XY analyses**
- ▼ **Column analyses**
 - t tests (and nonparametric tests)**
 - One-way ANOVA (and nonparametric o...)
 - One sample t and Wilcoxon test
 - Descriptive statistics
 - Normality and Lognormality Tests
 - Frequency distribution
 - ROC Curve
 - Bland-Altman method comparison
 - Identify outliers
 - Analyze a stack of P values
 - ▶ **Grouped analyses**
 - ▶ **Contingency table analyses**
 - ▶ **Survival analyses**
 - ▶ **Parts of whole analyses**
 - ▶ **Multiple variable analyses**
 - ▶ **Nested analyses**
 - ▶ **Generate curve**
 - ▶ **Simulate data**
 - ▶ **Recently used**

Analyze which data sets?

- A:male
- B:female

Select All

Deselect All



Cancel

OK

Experimental Design

Residuals

Options

Experimental design

 Unpaired Paired

	Group A	Group B
	Control	Treated
1	Y	Y
2		
3		
4		
5		

Assume Gaussian distribution?

 Yes. Use parametric test. No. Use nonparametric test.

Choose test

 Unpaired t test. Assume both populations have the same SD Unpaired t test with Welch's correction. Do not assume equal SDs

?

Cancel

OK

Parameters: t Tests (and Nonparametric Tests)

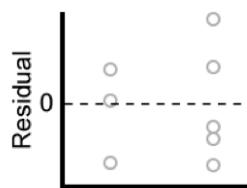
Experimental Design

Residuals

Options

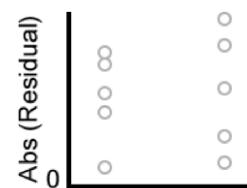
What graphs to create?

Correct model?



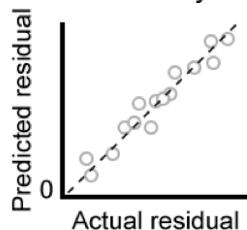
Residual plot

Equal variance?

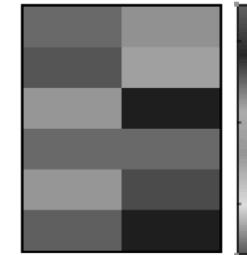


Homoscedasticity plot

Normality?



QQ plot



Heatmap plot

Diagnostics for residuals

Are the residuals Gaussian?

Normality tests of Anderson-Darling, D'Agostino, Shapiro-Wilk and Kolmogorov-Smirnov.

Make options on this tab be the default for future tests.



Cancel

OK

Parameters: t Tests (and Nonparametric Tests)

Experimental Design Residuals **Options**

Calculations

P value: One-tailed Two-tailed (recommended)

Report differences as: female - male

Confidence level: 95%

Definition of statistical significance: P < 0.05

Graphing options

Graph differences (paired)
 Graph ranks (nonparametric)
 Graph correlation (paired)
 Graph CI of difference between means

Additional results

Descriptive statistics for each dataset
 t Test: Also compare models using AICc
 Mann-Whitney: Also compute the CI of difference between medians
Assumes both distributions have the same shape.
 Wilcoxon: When both values on a row are identical, use method of Pratt
If this option is unchecked, those rows are ignored and the results will match prior version of Prism

Output

Show this many significant digits (for everything except P values): 4

P value style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), <0.000... N= 6

Make options on this tab be the default for future tests.

?

Cancel **OK**

temp_by_sex.pzfx — Edited

Search

▼ Data Tables

- Data 1
- + New Data Table...

▼ Info

- Project info 1
- + New Info...

▼ Results

- Unpaired t test of Data 1
- + New Analysis...

▼ Graphs

- Data 1
- QQ plot: Unpaired t test of Data 1
- Mean diff. CI plot: Unpaired t test
- + New Graph...

▼ Layouts

- + New Layout...

Family

▼ Data 1

- Unpaired t test
- QQ plot: Unpaired t test of Data 1
- Mean diff. CI plot: Unpaired t test

Tabular results

Unpaired t test

Tabular results

1	Table Analyzed	Data 1
2		
3	Column B	female
4	vs.	vs.
5	Column A	male
6		
7	Unpaired t test	
8	P value	0.0239
9	P value summary	*
10	Significantly different (P < 0.05)?	Yes
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=2.285, df=128
13		
14	How big is the difference?	
15	Mean of column A	98.10
16	Mean of column B	98.39
17	Difference between means (B - A)	0.2892 ± 0.1266
18	95% confidence interval	0.03882 to 0.5396
19	R squared (eta squared)	0.03921
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.132, 64, 64
23	P value	0.6211

Unpaired t test of Data 1

Row 1, Column A

temp_by_sex.pzfx — Edited

Search

Tabular results

Data Tables

- Data 1
- New Data Table...

Info

- Project info 1
- New Info...

Results

- Unpaired t test of Data 1
- New Analysis...

Graphs

- Data 1
- QQ plot: Unpaired t test of Data 1
- Mean diff. CI plot: Unpaired t test

Family

- Data 1
- Unpaired t test
- QQ plot: Unpaired t test of Data 1
- Mean diff. CI plot: Unpaired t test

Unpaired t test

Tabular results

20					
21	F test to compare variances				
22	F, DFn, Dfd	1.132, 64, 64			
23	P value	0.6211			
24	P value summary	ns			
25	Significantly different (P < 0.05)?	No			
26					
27	Normality of Residuals				
28	Test name	Statistics	P value	Passed normality test (alpha=0.05)?	P value summary
29	Anderson-Darling (A2*)	0.3633	0.4359	Yes	ns
30	D'Agostino-Pearson omnibus (K2)	2.467	0.2913	Yes	ns
31	Shapiro-Wilk (W)	0.9906	0.5264	Yes	ns
32	Kolmogorov-Smirnov (distance)	0.05178	0.1000	Yes	ns
33					
34	Data analyzed				
35	Sample size, column A	65			
36	Sample size, column B	65			
37					
38					

Unpaired t test of Data 1

Row 1, Column A

