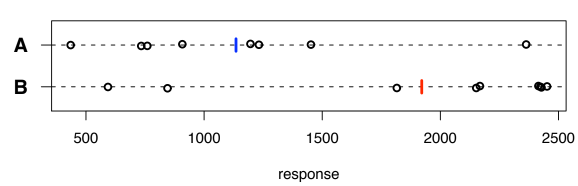
Inference about two groups

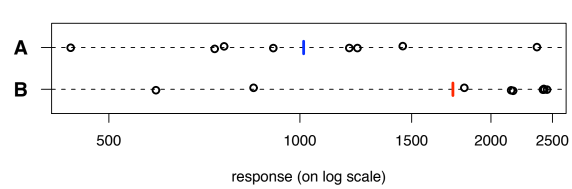
Differences between means

Suppose I measure the treatment response for 10 subjects getting treatment A and 10 subjects getting treatment B.

How different are the responses of the two treatments?

⟶ I am not interested in these *particular* subjects, but in the treatments *generally*.





$\overline{X} - \overline{Y}$

Suppose that

- $X_1, X_2, \ldots, X_n$ are iid Normal(mean=μ_A, SD=σ), and
- $Y_1, Y_2, \ldots, Y_m$ are iid Normal(mean=μ_B, SD=σ).

Then

$\longrightarrow$ $E(\overline{X} - \overline{Y}) = E(\overline{X}) - E(\overline{Y}) = \mu_A - \mu_B$

$\longrightarrow$ $SD(\overline{X} - \overline{Y}) = \sqrt{SD(\overline{X})^2 + SD(\overline{Y})^2} =$

$$\sqrt{\left(\frac{\sigma}{\sqrt{n}}\right)^2 + \left(\frac{\sigma}{\sqrt{m}}\right)^2} = \sigma\sqrt{\frac{1}{n} + \frac{1}{m}}$$

Note: If n = m, then $SD(\overline{X} - \overline{Y}) = \sigma\sqrt{2/n}$.

Pooled estimate of the population SD

We have two different estimates of the populations' SD, σ:

$$\hat{\sigma}_A = S_A = \sqrt{\frac{\sum(X_i - \overline{X})^2}{n-1}} \qquad \hat{\sigma}_B = S_B = \sqrt{\frac{\sum(Y_i - \overline{Y})^2}{m-1}}$$

We can use all of the data together to obtain an improved estimate of σ, which we call the "pooled" estimate.

$$\hat{\sigma}_{pooled} = \sqrt{\frac{\sum(X_i - \overline{X})^2 + \sum(Y_i - \overline{Y})^2}{n+m-2}}$$

$$= \sqrt{\frac{S_A^2(n-1) + S_B^2(m-1)}{n+m-2}}$$

Note: If n = m, then $\hat{\sigma}_{pooled} = \sqrt{\left(S_A^2 + S_B^2\right)/2}$

Estimated SE of $(\overline{X} - \overline{Y})$

$$\widehat{\text{SD}}(\overline{X} - \overline{Y}) = \hat{\sigma}_{\text{pooled}} \sqrt{\frac{1}{n} + \frac{1}{m}}$$

$$= \sqrt{\left[\frac{S_A^2(n-1) + S_B^2(m-1)}{n+m-2}\right] \cdot \left[\frac{1}{n} + \frac{1}{m}\right]}$$

In the case n = m,

$$\widehat{\text{SD}}(\overline{X} - \overline{Y}) = \sqrt{\frac{S_A^2 + S_B^2}{n}}$$

CI for the difference between the means

$$\frac{(\overline{X} - \overline{Y}) - (\mu_A - \mu_B)}{\widehat{\text{SD}}(\overline{X} - \overline{Y})} \sim t(\text{df} = n + m - 2)$$

The procedure:

1. Calculate $(\overline{X} - \overline{Y})$.
2. Calculate $\widehat{\text{SD}}(\overline{X} - \overline{Y})$.
3. Find the 97.5 percentile of the t distr'n with n + m – 2 d.f.
   $\longrightarrow$ t
4. Calculate the interval: $(\overline{X} - \overline{Y}) \pm t \cdot \widehat{\text{SD}}(\overline{X} - \overline{Y})$.

Example

Treatment A:

    2.67 2.86 2.87 3.04 3.09 3.09 3.13 3.27 3.35

$n = 9$, $\bar{x} \approx 3.04$, $s_A \approx 0.214$

Treatment B:

    3.78 3.06 3.64 3.31 3.31 3.51 3.22 3.67

$m = 8$, $\bar{y} \approx 3.44$, $s_B \approx 0.250$

$$\hat{\sigma}_{\text{pooled}} = \sqrt{\frac{s_A^2(n-1) + s_B^2(m-1)}{n+m-2}} = \ldots \approx 0.231$$

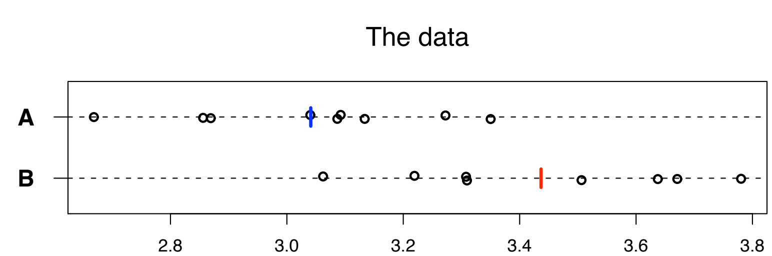
$$\widehat{\text{SD}}(\overline{X} - \overline{Y}) = \hat{\sigma}_{\text{pooled}} \sqrt{\frac{1}{n} + \frac{1}{m}} = \ldots \approx 0.112$$

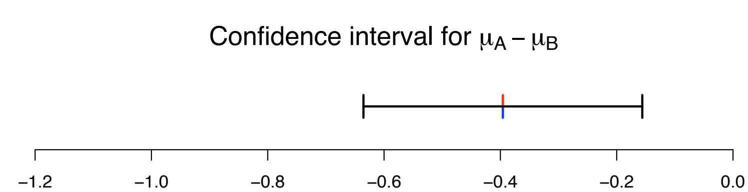
97.5 percentile of t(df=15) $\approx$ 2.13

Example

95% confidence interval:

$(3.04 - 3.44) \pm 2.13 \cdot 0.112 \;\approx\; -0.40 \pm 0.24 \;=\; (-0.64, -0.16).$





Example

Treatment A:

n = 10
sample mean: $\bar{x} = 55.22$
sample SD: $s_A = 7.64$
t value = `qt(0.975, 9)` = 2.26

$\longrightarrow$ 95% CI for μ_A:

$$55.22 \pm 2.26 \times 7.64 \,/\, \sqrt{10} \;=\; 55.2 \pm 5.5 \;=\; (49.8, 60.7)$$

Treatment B:

n = 16
sample mean: $\bar{x} = 68.2$
sample SD: $s_B = 18.1$
t value = `qt(0.975, 15)` = 2.13

$\longrightarrow$ 95% CI for μ_B:

$$68.2 \pm 2.13 \times 18.1 \,/\, \sqrt{16} \;=\; 68.2 \pm 9.7 \;=\; (58.6, 77.9)$$

Example

$$\hat{\sigma}_{\text{pooled}} = \sqrt{\tfrac{(7.64)^2 \times (10-1) + (18.1)^2 \times (16-1)}{10+16-2}} = 15.1$$

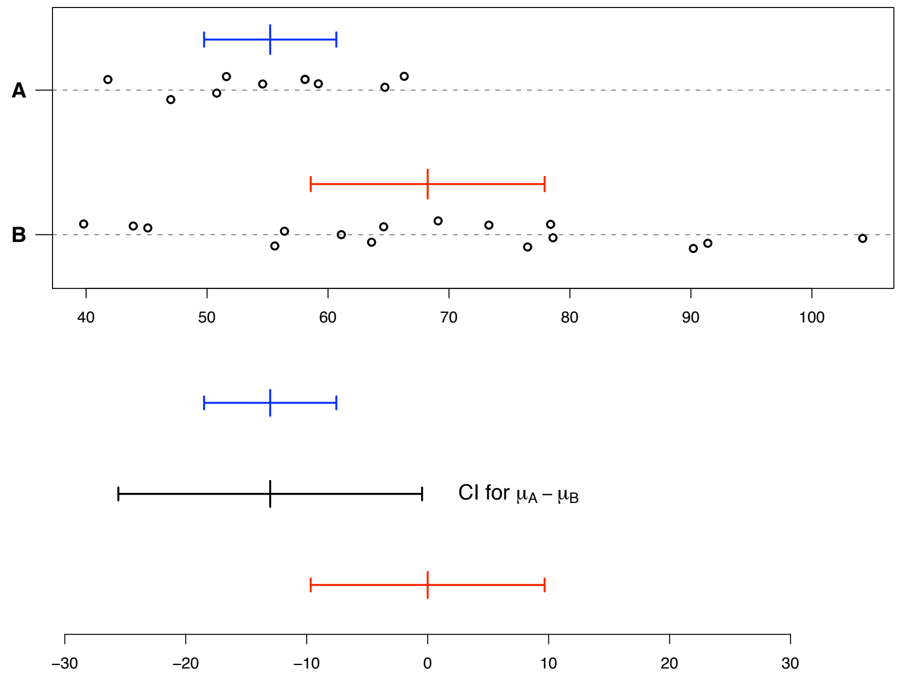
$$\widehat{\text{SD}}(\overline{X} - \overline{Y}) = \hat{\sigma}_{\text{pooled}} \times \sqrt{\tfrac{1}{n} + \tfrac{1}{m}} = 15.1 \times \sqrt{\tfrac{1}{10} + \tfrac{1}{16}} = 6.08$$

t value: `qt(0.975, 10+16-2)` = 2.06

$\longrightarrow$ 95% confidence interval for $\mu_A - \mu_B$:

$$(55.2 - 68.2) \pm 2.06 \times 6.08 \;=\; -13.0 \pm 12.6 \;=\; (-25.6, -0.5)$$

Example



One problem

What if the two populations really have different SDs, σ_A and σ_B?

Suppose that

- $X_1, X_2, \ldots, X_n$ are iid Normal(μ_A, σ_A),
- $Y_1, Y_2, \ldots, Y_m$ are iid Normal(μ_B, σ_B).

Then

$$\text{SD}(\overline{X} - \overline{Y}) = \sqrt{\frac{\sigma_A^2}{n} + \frac{\sigma_B^2}{m}} \qquad \widehat{\text{SD}}(\overline{X} - \overline{Y}) = \sqrt{\frac{S_A^2}{n} + \frac{S_B^2}{m}}$$

The problem:

$\longrightarrow$ $\dfrac{(\overline{X} - \overline{Y}) - (\mu_A - \mu_B)}{\widehat{\text{SD}}(\overline{X} - \overline{Y})}$ does not follow a t distribution.

An approximation

In the case that $\sigma_A \neq \sigma_B$:

Let $k = \dfrac{\left(\frac{s_A^2}{n} + \frac{s_B^2}{m}\right)^2}{\frac{(s_A^2/n)^2}{n-1} + \frac{(s_B^2/m)^2}{m-1}}$

Let $t^\star$ be the 97.5 percentile of the t distribution with k d.f.

$\longrightarrow$ Use $(\overline{X} - \overline{Y}) \pm t^\star \, \widehat{SD}(\overline{X} - \overline{Y})$ as a 95% confidence interval.

Example

$$k = \frac{[(7.64)^2/10 + (18.1)^2/16]^2}{\frac{[(7.64)^2/10]^2}{9} + \frac{[(18.1)^2/16]^2}{15}} = \frac{(5.84 + 20.6)^2}{\frac{(5.84)^2}{9} + \frac{(20.6)^2}{15}} = 21.8.$$

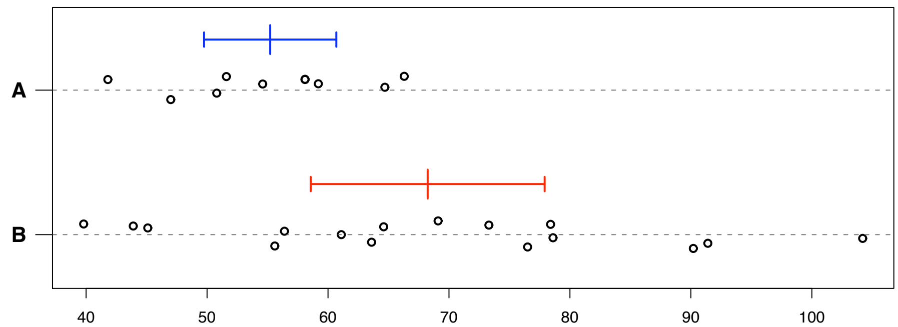
t value = `qt(0.975, 21.8)` = 2.07.

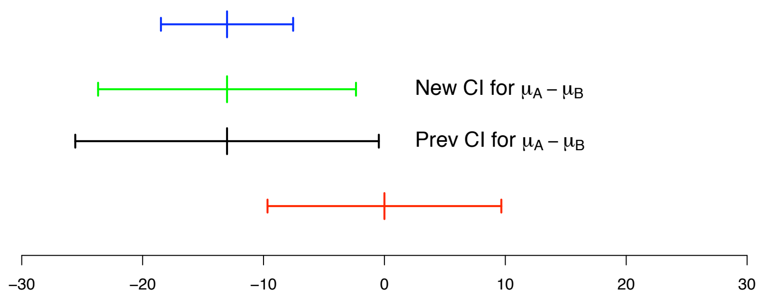
$$\widehat{SD}(\overline{X} - \overline{Y}) = \sqrt{\frac{s_A^2}{n} + \frac{s_B^2}{m}} = \sqrt{\frac{(7.64)^2}{10} + \frac{(18.1)^2}{16}} = 5.14.$$

$\longrightarrow$ 95% CI for $\mu_A - \mu_B$:

$$-13.0 \pm 2.07 \times 5.14 \quad = \quad -13.0 \pm 10.7 \quad = \quad (-23.7, -2.4)$$

Example





Degrees of freedom

○ One sample of size n:

$$X_1, X_2, \ldots, X_n \longrightarrow (\overline{X} - \mu)/(S/\sqrt{n}) \sim t(df = n - 1)$$

○ Two samples, of size n and m:

$$\begin{matrix} X_1, X_2, \ldots, X_n \\ Y_1, Y_2, \ldots, Y_m \end{matrix} \longrightarrow \frac{(\overline{X} - \overline{Y}) - (\mu_A - \mu_B)}{\hat{\sigma}_{\text{pooled}}\sqrt{\frac{1}{n} + \frac{1}{m}}} \sim t(df = n + m - 2)$$

What are these “degrees of freedom”?

Degrees of freedom

The degrees of freedom concern our estimate of the population standard deviation

We use the residuals $(X_1 - \overline{X}), \ldots, (X_n - \overline{X})$ to estimate σ.

⟶ But we really only have n – 1 independent data points ("degrees of freedom"), since $\sum(X_i - \overline{X}) = 0$.

In the two-sample case, we use $(X_1 - \overline{X}), (X_2 - \overline{X}), \ldots, (X_n - \overline{X})$ and $(Y_1 - \overline{Y}), \ldots, (Y_m - \overline{Y})$ to estimate σ.

⟶ But $\sum(X_i - \overline{X}) = 0$ and $\sum(Y_i - \overline{Y}) = 0$, and so we really have just n + m – 2 independent data points.

Testing the difference between two means

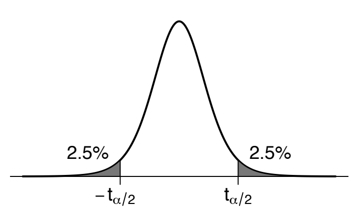
Treatment A: $X_1, \ldots, X_n \sim$ iid Normal(μ_A, σ_A)

Treatment B: $Y_1, \ldots, Y_m \sim$ iid Normal(μ_B, σ_B)

Test $H_0 : \mu_A = \mu_B$ vs $H_a : \mu_A \neq \mu_B$

Test statistic: $T = \dfrac{\overline{X} - \overline{Y}}{\sqrt{\frac{S_A^2}{n} + \frac{S_B^2}{m}}}$

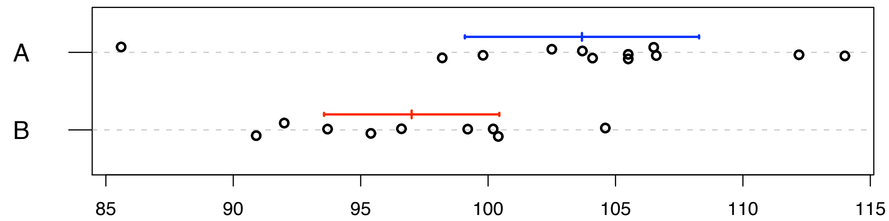
Reject H_0 if $|T| > t_{\alpha/2}$



If H_0 is true, then T follows (approximately) a t distr'n with k d.f.

k according to the nasty formula shown previously

Example



Treatment A: n = 12, sample mean = 103.7, sample SD = 7.2

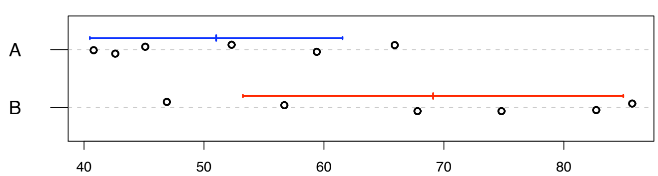
Treatment B: n = 9, sample mean = 97.0, sample SD = 4.5

$$\widehat{\mathrm{SD}}(\overline{X} - \overline{Y}) = \sqrt{\frac{7.2^2}{12} + \frac{4.5^2}{9}} = 1.80$$

T = (103.7 – 97.0)/1.80 = 2.60.

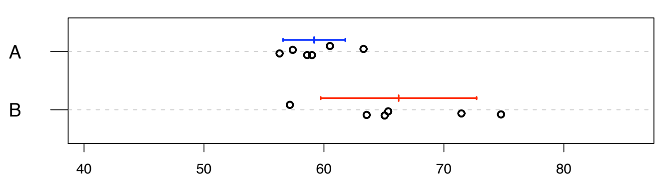
k = . . . = 18.48, so C = 2.10. Thus we reject H_0 at $\alpha = 0.05$.

Always give a confidence interval!



P = 0.019

95% CI: (–34.9, –1.2)

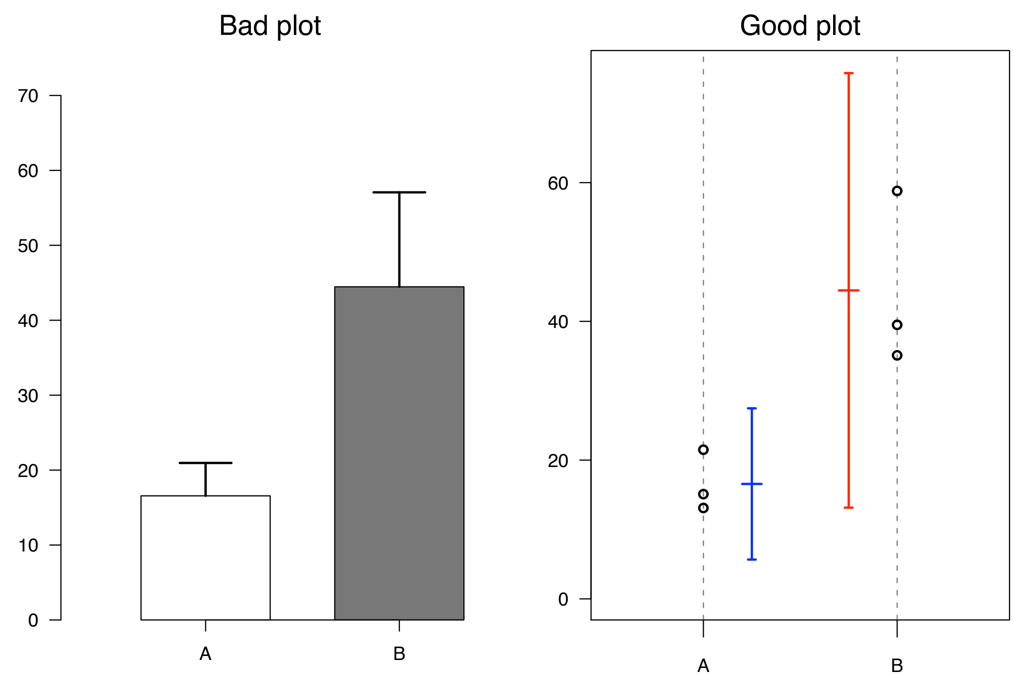


P = 0.019

95% CI: (–13.6, –0.5)

$\longrightarrow$ Make a statistician happy: draw a picture of the data.

Good plot, bad plot



What to say

When rejecting H_0:

- The difference is statistically significant.
- The observed difference can not reasonably be explained by chance variation.

When failing to reject H_0:

- There is insufficient evidence to conclude that $\mu_A \neq \mu_B$.
- The difference is not statistically significant.
- The observed difference could reasonably be the result of chance variation.

What about a different significance level?

Recall T = 2.60 k = 18.48

If α = 0.10, C = 1.73 $\Longrightarrow$ Reject H_0

If α = 0.05, C = 2.10 $\Longrightarrow$ Reject H_0

If α = 0.01, C = 2.87 $\Longrightarrow$ Fail to reject H_0

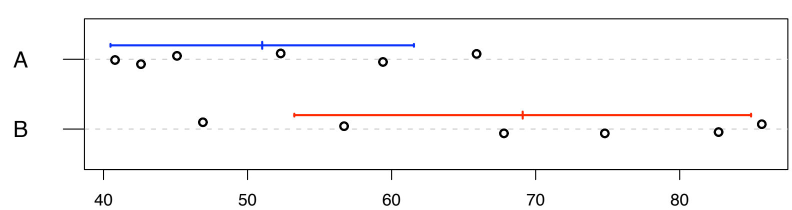
If α = 0.001, C = 3.90 $\Longrightarrow$ Fail to reject H_0

P-value: the smallest α for which you would still reject H_0 with the observed data.

With these data, P = `2*(1-pt(2.60,18.48))` = 0.018.

Another example

Suppose I measure the blood pressure of 6 subjects on a low salt diet and 6 subjects on a high salt diet. We wish to prove that the high salt diet causes an increase in blood pressure.



We imagine $X_1, \ldots, X_n \sim$ iid Normal(μ_L, σ_L) low salt

$Y_1, \ldots, Y_m \sim$ iid Normal(μ_H, σ_H) high salt

We want to test $H_0 : \mu_L = \mu_H$ versus $H_a : \mu_L < \mu_H$

$\longrightarrow$ Are the data compatible with H_0?

A one-tailed test

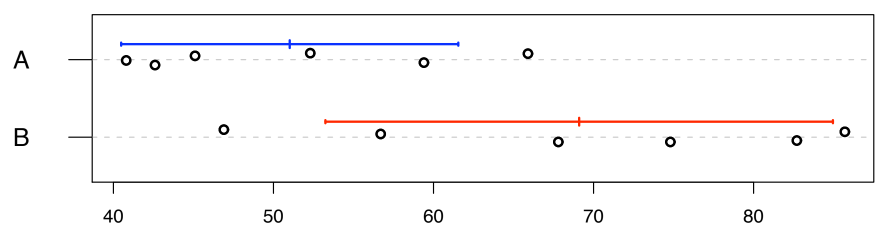
Test statistic: T = $\dfrac{\overline{X} - \overline{Y}}{\widehat{\text{SD}}(\overline{X} - \overline{Y})}$

Since we seek to prove that μ_L is smaller than μ_H, only large negative values of the statistic are interesting.

Thus, our rejection region is T $<$ C for some critical value C.

We choose C so that Pr(T $<$ C $|$ $\mu_L = \mu_H$) = α.

The example



Low salt: n = 6; sample mean = 51.0, sample SD = 10.0

High salt: n = 6; sample mean = 69.1, sample SD = 15.1

$\bar{x} - \bar{y}$ = –18.1 $\quad\quad$ $\widehat{\text{SD}}(\overline{X} - \overline{Y})$ = 7.40 $\quad\quad$ T = –18.1 / 7.40 = –2.44

k = 8.69. If α = 0.05, then C = –1.84.

Since T $<$ C, we reject H_0 and conclude that $\mu_L < \mu_H$.

Note: P-value = `pt(-2.44, 8.69)` = 0.019.

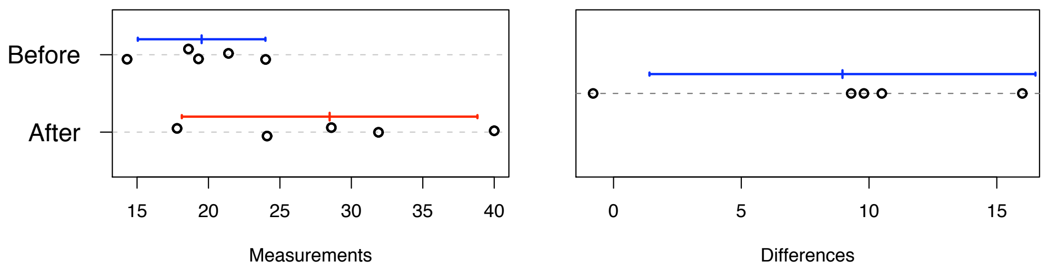
Example

Suppose I do some pre/post measurements.

I make some measurement on each of 5 subjects before and after some treatment.

Question: Does the treatment have any effect?

Subject	1	2	3	4	5
Before	18.6	14.3	21.4	19.3	24.0
After	17.8	24.1	31.9	28.6	40.0



Pre/post example

In this sort of pre/post measurement example, study the differences as a single sample.

Why? The pre/post measurements are likely associated, and as a result one can more precisely learn about the effect of the treatment.

Subject	1	2	3	4	5
Before	18.6	14.3	21.4	19.3	24.0
After	17.8	24.1	31.9	28.6	40.0
Difference	–0.8	9.8	10.5	9.3	16.0

n = 5; mean difference = 8.96; SD difference = 6.08.

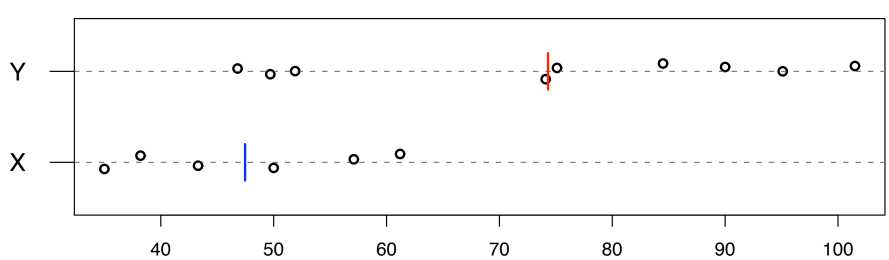
95% CI for underlying mean difference = . . . = (1.4, 16.5)

P-value for test of $\mu_{\text{before}} = \mu_{\text{after}}$: 0.03.

Summary

- Tests of hypotheses → answering yes/no questions regarding population parameters.
- There are two kinds of errors:
  - Type I: Reject H_0 when it is true.
  - Type II: Fail to reject H_0 when it is false.
- If we fail to reject H_0, we do not "accept H_0".
- P-value: the probability, if H_0 is true, of obtaining data as extreme as was observed. Pr(data | no effect) rather than Pr(no effect | data).
- P-values are a function of the data (and thus, they are random).
- Power: the probability of rejecting H_0 when it is false.
- Always look at the confidence interval as well as the P-value.

Example



$\overline{X} = 47.5$ $\quad s_A = 10.5$ $\quad n = 6$

$\overline{Y} = 74.3$ $\quad s_B = 20.6$ $\quad m = 9$

$s_p = 17.4$ $\quad T = -2.93$

$\longrightarrow$ P = `2*pt(-2.93,6+9-2)` = 0.011.

Wilcoxon rank-sum test

Rank the X's and Y's from smallest to largest (1, 2, . . . , n+m)

R = sum of ranks for X's

(Also known as the Mann-Whitney Test)

X	Y	rank
35.0		1
38.2		2
43.3		3
	46.8	4
	49.7	5
50.0		6
	51.9	7
57.1		8
61.2		9
	74.1	10
	75.1	11
	84.5	12
	90.0	13
	95.1	14
	101.5	15

R = 1 + 2 + 3 + 6 + 8 + 9 = 29

P-value = 0.026

Note: The distribution of R (given that X's and Y's have the same dist'n) is calculated numerically

Permutation test

X or Y	group
X_1	1
X_2	1
$\vdots$	1
X_n	1
Y_1	2
Y_2	2
$\vdots$	2
Y_m	2

$\longrightarrow T_{obs}$

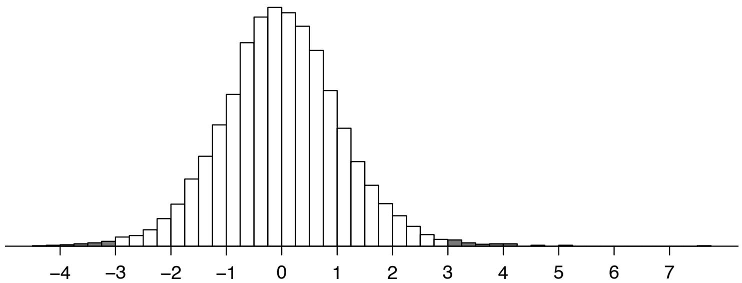
X or Y	group
X_1	2
X_2	2
$\vdots$	1
X_n	2
Y_1	1
Y_2	2
$\vdots$	1
Y_m	1

$\longrightarrow T^{\star}$

Group status shuffled

Compare the observed t-statistic to the distribution obtained by randomly shuffling the group status of the measurements.

Permutation distribution



P-value = $\Pr(|T^{\star}| \geq |T_{obs}|)$

$\longrightarrow$ Small n & m: Look at all $\binom{n+m}{n}$ possible shuffles

$\longrightarrow$ Large n & m: Look at a sample (w/ repl) of 1000 such shuffles

Example data:

All 5005 permutations: P = 0.015; sample of 1000: P = 0.013.

Estimating the permutation P-value

Let P be the true P-value (if we do *all possible* shuffles).

Do N shuffles, and let X be the number of times the statistic after shuffling is bigger or equal to the observed statistic.

$\longrightarrow$ $\hat{P} = \frac{X}{N}$ where $X \sim$ Binomial(N,P)

$\longrightarrow$ $E(\hat{P}) = P$ $\qquad SD(\hat{P}) = \sqrt{\frac{P(1-P)}{N}}$

If the “true” P-value was P = 5%, and we do N=1000 shuffles:

$SD(\hat{P})$ = 0.7%.

Summary

The t-test relies on a normality assumption.
If this is a worry, consider:

- Paired data:
  - Sign test
  - Signed rank test
  - Permutation test
- Unpaired data:
  - Rank-sum test
  - Permutation test

$\longrightarrow$ The crucial assumption is independence!

The fact that the permutation distribution of the t-statistic is often closely approximated by a t distribution is good support for just doing t-tests.

Maximum Likelihood Estimation

Estimation

Goal: Estimate a population parameter θ.

Data: $X_1, X_2, \ldots, X_n \sim$ iid with distribution depending on θ.

If one has many estimators to choose from, pick

- That with the smallest SE, among all unbiased estimators
- That with the smallest RMS error, even if biased

$\longrightarrow$ Sometimes it is not clear how to form even one good estimator.

Maximum likelihood estimation

Likelihood function: $L(\theta) = \Pr(\text{data} \mid \theta)$

Log likelihood: $l(\theta) = \log \Pr(\text{data} \mid \theta)$

Maximum likelihood estimate:

Choose, as the estimate of θ, the value of θ for which the likelihood function $L(\theta)$ (or equivalently, the log likelihood function) is maximized.

$\longrightarrow$ You need to solve these equations analytically or numerically.

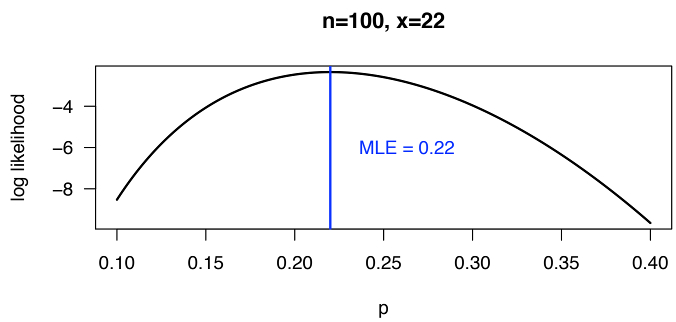
Example 1

Suppose $X \sim \text{Binomial}(n, p)$.

log likelihood function: $l(p) = \log\left\{\binom{n}{x} p^x (1-p)^{(n-x)}\right\}$

$$= x \log(p) + (n-x)\log(1-p) + \text{constant}$$

MLE: the obvious thing: $\hat{p} = x/n$



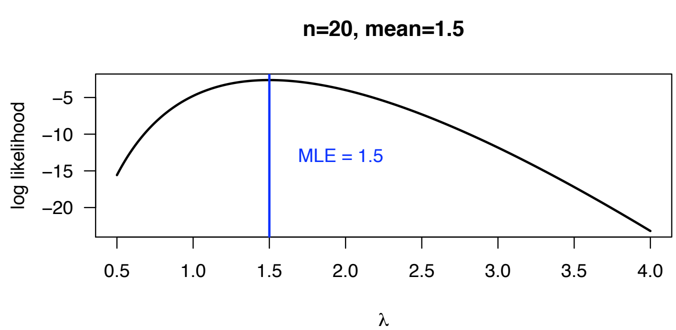
Example 2

Suppose $X_1, \ldots, X_{20} \sim$ iid Poisson(λ).

log likelihood function: $l(\lambda) = \log\left\{\prod_i e^{-\lambda} \lambda^{x_i}/x_i!\right\}$

$$= \ldots = -20\lambda + \left(\sum x_i\right)\log\lambda + \text{ constant}$$

MLE: the obvious thing: $\hat{\lambda} = \bar{x}$



Example 3

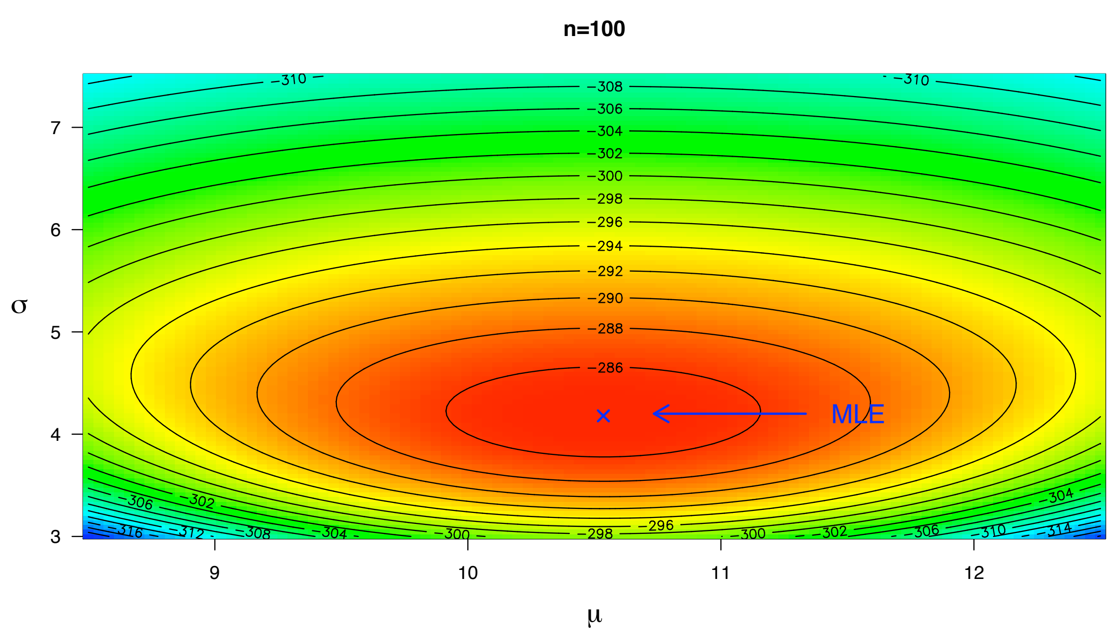
Suppose $X_1, \ldots, X_n \sim$ iid $N(\mu, \sigma)$

log likelihood function: $l(\mu, \sigma) = \log \left\{ \prod_i \frac{1}{\sigma\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{x-\mu}{\sigma} \right)^2 \right] \right\}$

MLEs: almost the obvious things:

$$\hat{\mu} = \bar{x} \qquad \hat{\sigma} = \sqrt{\sum (x_i - \bar{x})^2 / n}$$

Example 3: the log likelihood surface



About MLEs

Maximum likelihood estimation is a general procedure for finding a reasonable estimator

- In many cases, the MLE turns out to be the obvious thing.
- MLEs are often very good (but not necessarily the best) possible estimators:
  - unbiased or nearly unbiased
  - small standard errors
- Sometimes obtaining the MLEs requires hefty computation!

Example 4: ABO blood groups

Phenotype	**Genotype**	**Frequency**
O	OO	p_O^2
A	AA or AO	$p_A^2 + 2p_Ap_O$
B	BB or BO	$p_B^2 + 2p_Bp_O$
AB	AB	$2p_Ap_B$

Frequencies under the assumption of Hardy-Weinberg equilibrium.

Example 4: Data

Phenotype	No. subjects	% subjects
O	117	46.8%
A	98	39.2%
B	29	11.6%
AB	6	2.4%
Total	**250**	**100%**

$\longrightarrow$ What are the estimates of p_A, p_B, p_O?

Example 4: Estimates

Simple estimates:

$\longrightarrow$ $\tilde{p}_O = \sqrt{0.468} = 0.684$

$\longrightarrow$ $\tilde{p}_A^2 + 2\tilde{p}_A 0.684 = 0.392 \longrightarrow \tilde{p}_A = 0.243$

$\longrightarrow$ $\tilde{p}_B = 0.024/(2\tilde{p}_A) = 0.072$

Log likelihood:

$$l(p_O, p_A, p_B) =$$

$$117 \log(p_O^2) + 98 \log(p_A^2 + 2p_A p_O) + 29 \log(p_B^2 + 2p_B p_O) + 6 \log(2p_A p_B)$$

Example 5: log likelihood