Sections 7.1 and 7.2

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Stat 205: Elementary Statistics for the Biological and Life Sciences

Hypothesis testing

- Often scientists wish to test a hypothesis, or statement of fact.
- **Example 7.2.1** scientists wish to test the hypothesis that norepinephrine (NE) levels are different in rats exposed to toluene (glue) and those that aren't.
- The scientist designs a controlled experiment in which $n_1 = 6$ rats are exposed to toluene and $n_2 = 5$ rats are not. NE levels are measured in the rats' brains.
- The scientists wish to show that the population NE levels are different among rats exposed and non-exposed to toluene.
- This is encapsulated in the mathematical statement $H_A : \mu_1 \neq \mu_2$, the *mean* NE levels differ across exposed and non-exposed.

Hypothesis testing

- A hypothesis test is a proof by contradiction.
- We *assume* the null $H_0 : \mu_1 = \mu_2$ is true, then the data shows us something that is absurd, casting doubt on what we assumed, namely $H_0 : \mu_1 = \mu_2$.
- So we have to conclude the opposite, $H_A : \mu_1 \neq \mu_2$.
- The **null hypothesis** is *what we are trying to disprove*, $H_0 : \mu_1 = \mu_2$.
- The **alternative hypothesis** is *what we're trying to show is true*, $H_A : \mu_1 \neq \mu_2$.

Hypothesis testing

- Recall from Chapter 6, that if data are normal in both populations then

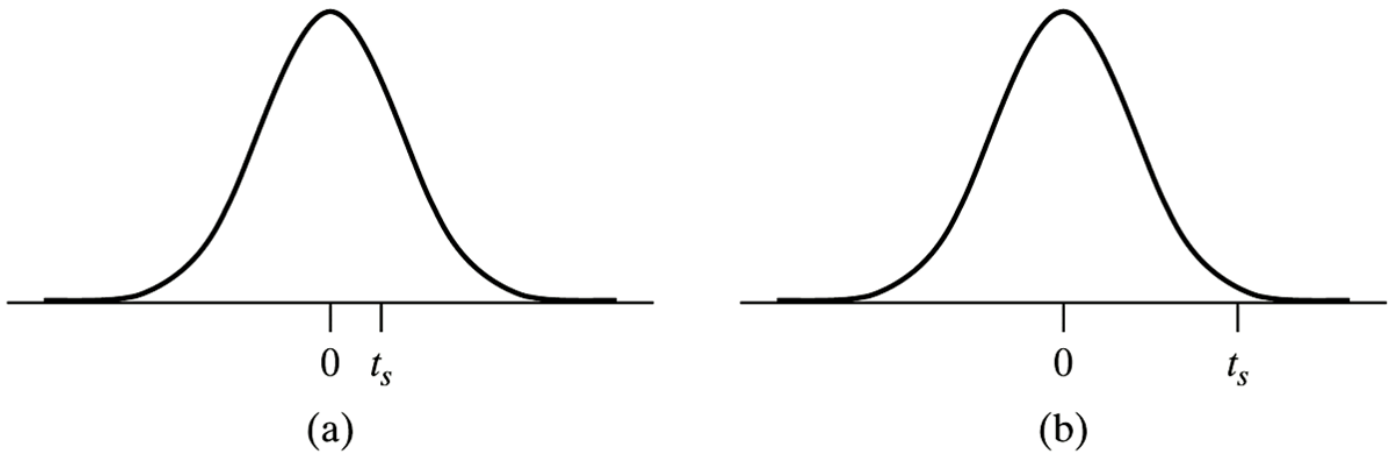
$$t_s = \frac{\bar{Y}_1 - \bar{Y}_2 - (\mu_1 - \mu_2)}{SE_{\bar{Y}_1 - \bar{Y}_2}}$$

  has a t distribution with df given by the Satterthwaite-Welch formula.
- In the hypothesis test, we are *assuming* $\mu_1 - \mu_2 = 0$, so

$$t_s = \frac{\bar{Y}_1 - \bar{Y}_2}{SE_{\bar{Y}_1 - \bar{Y}_2}}$$

  has a t distribution, which is centered at zero.
- If t_s is really far away from zero in either direction, we have evidence that $H_0 : \mu_1 = \mu_2$ is *not true*.
- $|t_s|$ measures how far apart $\bar{Y}_1$ and $\bar{Y}_2$ are, i.e. how many $SE_{\bar{Y}_1 - \bar{Y}_2}$'s apart.

t test schematic



(a) Data compatible with H_0 (so no evidence toward H_A), (b) data not compatible with H_0 (in favor of H_A).

Example 7.2.1 Parallel dotplots NE concentration

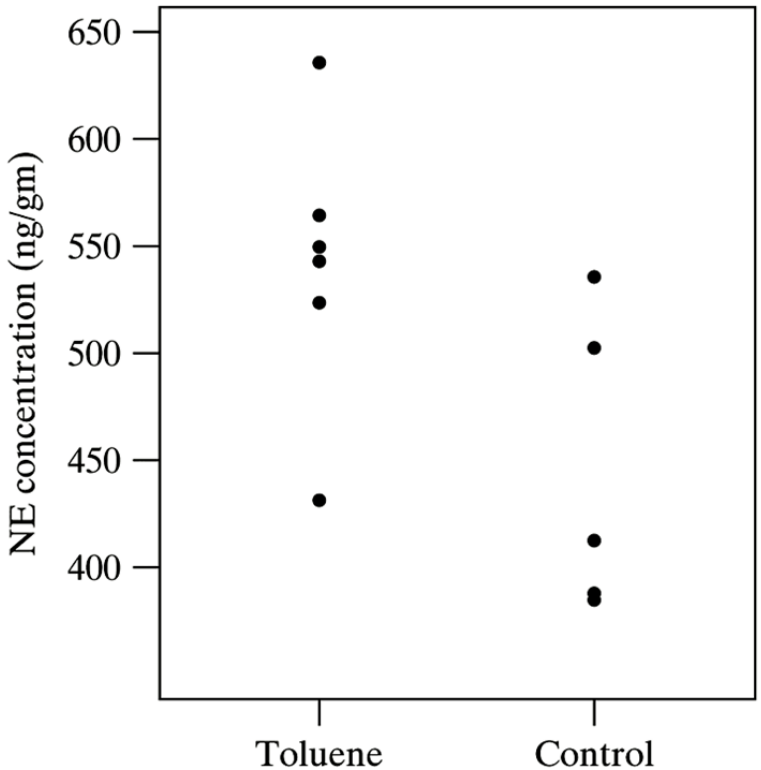


Figure 7.2.1 Parallel dotplots of NE concentration

Normality okay? Does there appear to be a mean difference?

Example 7.2.2 NE concentration (ng/gm)

Table 7.2.1 NE concentration (ng/gm)

	Toluene (Group 1)	Control (Group 2)
	543	535
	523	385
	431	502
	635	412
	564	387
	549	
n	6	5
$\bar{y}$	540.8	444.2
s	66.1	69.6
SE	27	31

$$t_s = \frac{\bar{y}_1 - \bar{y}_2}{SE_{\bar{Y}_1 - \bar{Y}_2}} = \frac{540.8 - 444.2}{\sqrt{\frac{66.1^2}{6} + \frac{69.6^2}{5}}} = 2.34.$$

$\bar{y}_1$ is 2.34 SE's away from $\bar{y}_2$. This is big, but how big?

P-values

- The P-**value** for a hypothesis test is the probability of the test statistic being at least as extreme as the observed test statistic, *assuming* H_0 *is true*.
- P-value answers the question "how big is big?" for $|t_s|$.

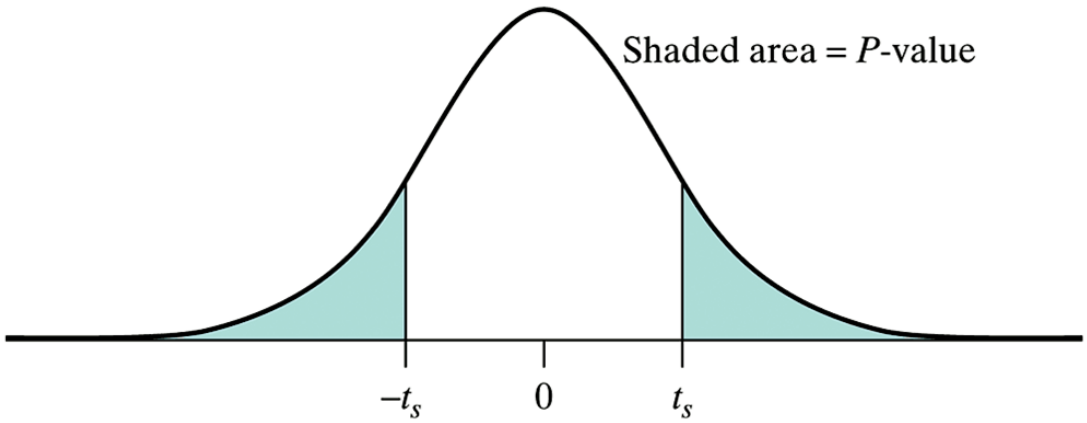
P-value for two-sample problem

- For the two-sample problem, P-value is the probability of seeing *sample means* $\bar{Y}_1$ *and* $\bar{Y}_2$ *even further apart than what we saw, if* $H_0 : \mu_1 = \mu_2$ *is true.*
- This is a standard probability calculation due to W.S. Gosset

$$\begin{aligned}
\text{P-value} &= \Pr\{|\bar{Y}_1 - \bar{Y}_2| > |\bar{y}_1 - \bar{y}_2| | H_0 \text{ true}\} \\
&= \Pr\left\{\left|\frac{\bar{Y}_1 - \bar{Y}_2}{SE_{\bar{Y}_1 - \bar{Y}_2}}\right| > \left|\frac{\bar{y}_1 - \bar{y}_2}{SE_{\bar{Y}_1 - \bar{Y}_2}}\right| | H_0 \text{ true}\right\} \\
&= \Pr\{|T_{df}| > |t_s|\}
\end{aligned}$$

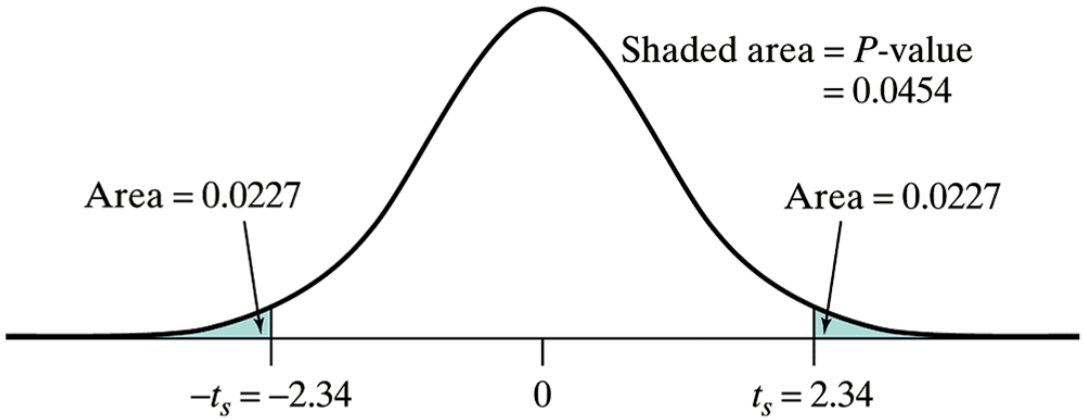
where T_{df} is a t random variable with df given by the Satterthwaite-Welch formula.

Two-tailed p-value for the t test



- The P-value is computed in R using t.test(data1,data2).
- It is the area in the tails of the t distribution with Satterthwaite-Welch *df*.

Example 7.2.3 Two-tailed P-value toluene data




```
> toluene=c(543,523,431,635,564,549)
> control=c(535,385,502,412,387)
> t.test(toluene,control)

        Welch Two Sample t-test

data:  toluene and control
t = 2.3447, df = 8.451, p-value = 0.04543
alternative hypothesis: true difference in means is not equal to 0
```

Note that $t_s = 2.34$, just as we computed by hand. What df are being used?

What do we decide based on the P-value?

- $|t_s|$ is "big" when the P-value is "small." But how small is small?
- We compare the P-value to a cutoff value denoted α.
- α is called the **significance level** of the hypothesis test; it is almost always $\alpha = 0.05$.
- If P-value $< \alpha$ then we **reject** H_0 at significance level α.
- If P-value $> \alpha$ then we **accept** H_0 at significance level α.
- Some books demand that you say "do not reject H_0" instead of "accept H_0."
- α has an interpretation that we'll talk about next time.

Example 7.2.4 NE concentration data

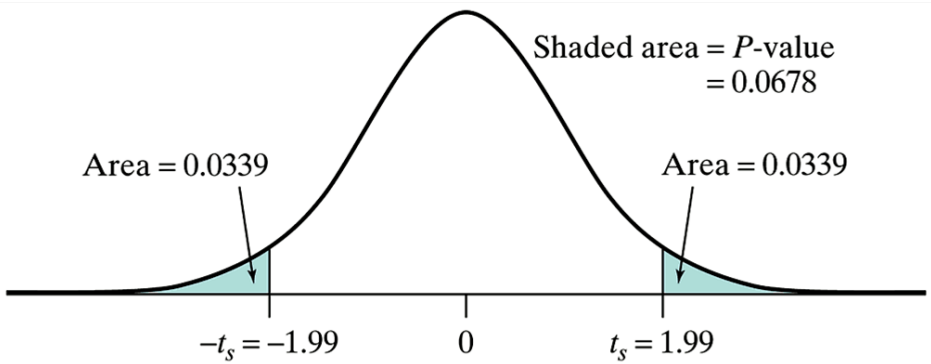
```
> toluene=c(543,523,431,635,564,549)
> control=c(535,385,502,412,387)
> t.test(toluene,control)

        Welch Two Sample t-test

data:  toluene and control
t = 2.3447, df = 8.451, p-value = 0.04543
alternative hypothesis: true difference in means is not equal to 0
```

We reject $H_0 : \mu_1 = \mu_2$ at the 5% significance level because P-value $= 0.0454 < 0.05 = \alpha$. There is statistically significant evidence that the mean norepinephrine levels are different in toluene-exposed vs. non-exposed rats.

Example 7.2.5 Two-week height of control & ancy plants




```
> control=c(10.0,13.2,19.8,19.3,21.2,13.9,20.3,9.6)
> ancy=c(13.2,19.5,11.0,5.8,12.8,7.1,7.7)
> t.test(control,ancy)

t = 1.9939, df = 12.783, p-value = 0.06795
alternative hypothesis: true difference in means is not equal to 0
```

As P-value $= 0.068 > 0.05 = \alpha$, we accept $H_0 : \mu_1 = \mu_2$ at the 5% level. There is no evidence that the mean heights are different in control vs. ancy populations.

Assumptions behind two-sample t test

- If the sample sizes are large enough ($n_1 > 30$ and $n_2 > 30$, say) the t-test works okay because of the central limit theorem.
- If the sample sizes are small, data from each population *needs to be normal* for the procedure to work okay. If not, we can't trust the t-test P-value.
- When sample sizes are small and data are not normal, there is an alternative method to compute the P-value that *doesn't assume anything* about the population shapes.
- This approach is called a randomized test or **permutation test**, and uses *resampling methods*.
- Resampling methods are a powerful approach to statistics and include permutation tests and bootstrapping.

Example 7.1.1

- An exercise science researcher studied the trunk flexion flexibility (cm) of $n_1 = 4$ women in an aerobics class, and $n_3 = 3$ women who were dancers.
- Among aerobics class participants we have 38, 45, 58, and 64 cm.
- Among dancers we have 48, 59, and 61 cm.
- Is there a difference in $\mu_1 =$ population mean stretching of aerobics vs. $\mu_2 =$ population mean stretching of dancers?
- If there *truly is no difference*, then all 7 observations *came from the same population distribution*.
- If there *truly is no difference*, then all arrangements of $n_1 = 3$ observations and $n_2 = 4$ observations are *equally likely*.

Permutation test of $H_0 : \mu_1 = \mu_2$

- Compute the observed test statistic $d_s = \bar{y}_1 - \bar{y}_2$.
- Consider all possible arrangements of $n_1 = 4$ and $n_2 = 3$ and compute the mean differences d from these. The histogram from this is called the **permutation density**. This is the distribution of the test statistic assuming $H_0 : \mu_1 = \mu_2$ is true.
- The P-value for $H_0 : \mu_1 = \mu_2$ vs. $H_A : \mu_1 \neq \mu_2$ is the proportion of $|d|$'s bigger than $|d_s|$.

Permutation samples

Table 7.1.2

Sample 1 ("aerobics")	Sample 2 ("dance")	Mean of sample 1	Mean of sample 2	Difference in means
38 45 58 64	48 59 61	51.25	56.00	**−4.75**
38 45 58 48	64 59 61	47.25	61.33	**−14.08**
38 45 58 59	64 48 61	50.00	57.67	**−7.67**
38 45 58 61	64 48 59	50.50	57.00	**−6.50**
38 45 64 48	58 59 61	48.75	59.33	**−10.58**
38 45 64 59	58 48 61	51.50	55.67	**−4.17**
38 45 64 61	58 48 59	52.00	55.00	**−3.00**
38 45 48 59	58 64 61	47.50	61.00	**−13.50**
38 45 48 61	58 64 59	48.00	60.33	**−12.33**
38 45 59 61	58 64 48	50.75	56.67	**−5.92**
38 58 64 48	45 59 61	52.00	55.00	−3.00
38 58 64 59	45 48 61	54.75	51.33	3.42
38 58 64 61	45 48 59	55.25	50.67	4.58
38 58 48 59	45 64 61	50.75	56.67	**−5.92**
38 58 48 61	45 64 59	51.25	56.00	**−4.75**

(*Continues on next page*)

10 $|d|$'s are bigger than $|d_s| = 4.75$ out of the first 15 possible combinations.

Permutation samples cont'd

Table 7.1.2 (Continued)

Sample 1 ("aerobics")	Sample 2 ("dance")	Mean of sample 1	Mean of sample 2	Difference in means
38 58 59 61	45 64 48	54.00	52.33	1.67
38 64 48 59	45 58 61	52.25	54.67	−2.42
38 64 48 61	45 58 59	52.75	54.00	−1.25
38 64 59 61	45 58 48	55.50	50.33	**5.17**
38 48 59 61	45 58 64	51.50	55.67	−4.17
45 58 64 48	38 59 61	53.75	52.67	1.08
45 58 64 59	38 48 61	56.50	49.00	**7.50**
45 58 64 61	38 48 59	57.00	48.33	**8.67**
45 58 48 59	38 64 61	52.50	54.33	−1.83
45 58 48 61	38 64 59	53.00	53.67	−0.67
45 58 59 61	38 64 48	55.75	50.00	**5.75**
45 64 48 59	38 58 61	54.00	52.33	1.67
45 64 48 61	38 58 59	54.50	51.67	2.83
45 64 59 61	38 58 48	57.25	48.00	**9.25**
45 48 59 61	38 58 64	53.25	53.33	−0.08
58 64 48 59	38 45 61	57.25	48.00	**9.25**
58 64 48 61	38 45 59	57.75	47.33	**10.42**
58 64 59 61	38 45 48	60.50	43.67	**16.83**
58 48 59 61	38 45 64	56.50	49.00	**7.50**
64 48 59 61	38 45 58	58.00	47.00	**11.00**

10 $|d|$'s are bigger than $|d_s| = 4.75$ out of the last 20 possible combinations.

Final P-value

There are 20 $|d|$'s bigger than $|d_s|$ out of 35 possible combinations. The P-value is $\frac{20}{35} = 0.57$.

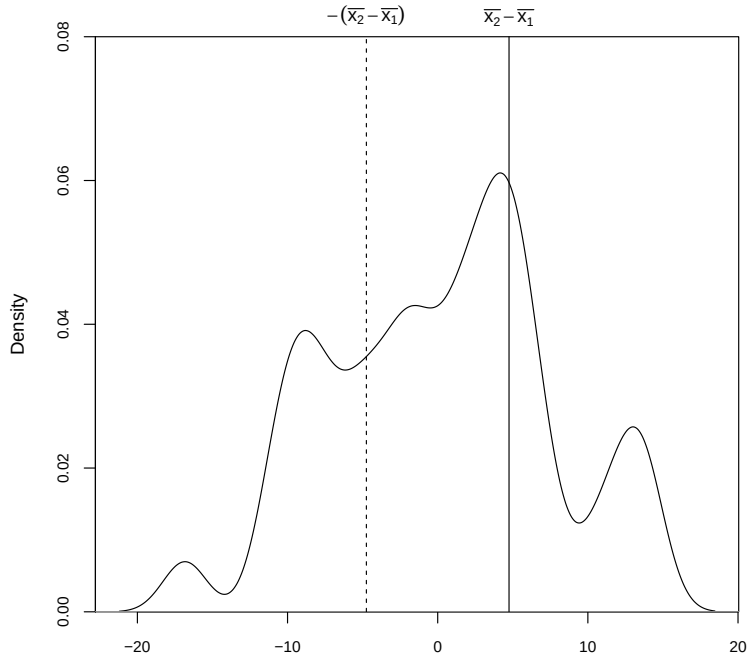
We accept $H_0 : \mu_1 = \mu_2$ at the 5% level because P-value $= 0.54 > 0.05 = \alpha$. There is no evidence that the mean trunk flexion is different between dancers and aerobics participants.

DAAG package in R

- Many people have written specialized R code to carry out non-standard analyses.
- The `DAAG` (Data Analysis And Graphics data and functions) package has a function to do a two-sample permutation test as described in the book.
- The function works the same as t.test, e.g. `twot.permutation(data1,data2)`.
- You will get a P-value and an estimate of the permutation density (based on an approximation).
- In R click `Packages`, then `Install package(s)...`, then pick a mirror (a place to download from – any will work), then scroll down until you find `DAAG` and install it.
- After installing it, you need to load it. Under `Packages` pick `Load package...`, then pick out `DAAG`.

Trunk flexion in R

```
> aerobics=c(38,45,58,64)
> dance=c(48,59,61)
> twot.permutation(aerobics,dance)
[1] 0.572
```



The P-value is the area outside of $-d_s$ an d_s.

Comparing permutation test to t-test

```
> control=c(10.0,13.2,19.8,19.3,21.2,13.9,20.3,9.6)
> ancy=c(13.2,19.5,11.0,5.8,12.8,7.1,7.7)
> twot.permutation(control,ancy)
[1] 0.08
> t.test(control,ancy)
t = 1.9939, df = 12.783, p-value = 0.06795

> aerobics=c(38,45,58,64)
> dance=c(48,59,61)
> twot.permutation(aerobics,dance)
[1] 0.572
> t.test(aerobics,dance)
t = -0.6615, df = 4.86, p-value = 0.5384
```

The permutation-test and t-test P-values are similar in both cases.
Remember: the permutation test *can always be used*, even in small samples with non-normal data.

Ingredients of a two-sided hypothesis test $H_0 : \mu_1 = \mu_2$

- Clearly define the population means μ_1 and μ_2. Choose a significance level α (usually $\alpha = 0.05$).
- State the null hypothesis $H_0 : \mu_1 = \mu_2$ and the alternative hypothesis $H_A : \mu_1 \neq \mu_2$.
- If $n_1 < 30$ or $n_2 < 30$ then check if data are normal using normal probability plots and/or dotplots. If data are approximately normal then do t-test, otherwise do permutation-test (or Mann-Whitney-Wilcoxin test, later...)
- R computes t_s, df, and the P-value from $\bar{y}_1$, $\bar{y}_2$, s_1, s_2, n_1, and n_2 using the `t.test` function. For small sample sizes and non-normal data use `twot.permutation` or `wilcox.test`.
- All tests give a P-value. Compare P-value to α; if P-value$< \alpha$ then reject $H_0 : \mu_1 = \mu_2$, otherwise accept H_0.
- State conclusion; e.g. "P-value $= 0.068 > 0.05 = \alpha$, accept $H_0 : \mu_1 = \mu_2$ at the 5% level. There is no evidence that mean heights are different in control vs. ancy populations."