Topic 6

Classical Statistical Tests for Oneand Two-Sample Cases

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6. Classical Statistical Tests for Oneand Two-Sample Cases

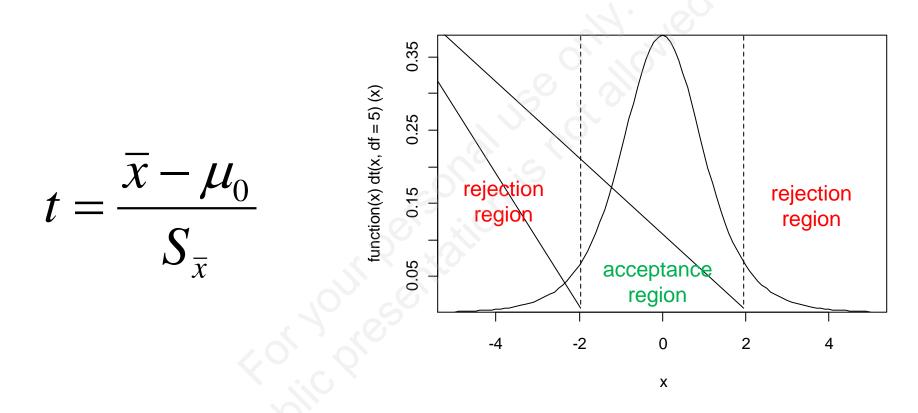
6.1. One-sample t-test

Null hypothesis of a one-sample t-test

H₀: the sample of interest comes from a normally distributed population with mean μ_0

i.e. $H_0: \mu = \mu_0$

Student t for one-sample case



- If *t* falls outside the acceptance region, reject H₀
- Alternatively, calculate the probability of obtaining a *t*-value as large or larger than the observed *t*-value. If P < 0.05, reject H₀

An example...

Daily energy intake (kJ/day) in 11 women: > d.intake <- c(5260, 5470, 5640, 6180, 6390, 6515, 6805, 7515, 7515, 8230, 8770) > mean(d.intake) [1] 6753.6 > sd(d.intake) [1] 1142.1

Does the sample mean differ significantly from a norm of 7725 kJ/day?

```
# One-sample t-test:
> t.test(d.intake, mu = 7725)
```

```
One Sample t-test
```

```
data: d.intake
t = -2.8208, df = 10, p-value = 0.01814
alternative hypothesis: true mean is not equal to 7725
95 percent confidence interval:
 5986.348 7520.925
sample estimates:
mean of x
 6753.636
```

6. Classical Statistical Tests for Oneand Two-Sample Cases

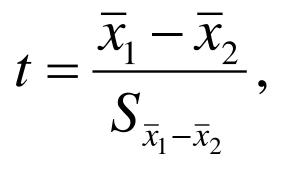
6.2. Two-sample t-test

Null hypothesis of a two-sample t-test

H₀: two samples come from normal distributions with the same mean

i.e.
$$H_0: \mu_1 = \mu_2$$

Student t for a two-sample case



 $t = \frac{x_1 - x_2}{S_{\bar{x}_1 - \bar{x}_2}}, \quad where \quad S_{\bar{x}_1 - \bar{x}_2} = \sqrt{S_{\bar{x}_1} - S_{\bar{x}_2}}$

Only if group variances don't differ. If they do, the Welch approximate test is used

- Again, if t falls outside the acceptance region, reject H₀
- Alternatively, calculate the probability of obtaining a *t*-value as large or larger than the observed *t*-value. If P < 0.05, reject H₀

An example...

- # Datasets that accompany the book of Peter Dalgaard (2008):
- > library(ISwR)
- # Data on daily energy expenditure in lean and obese women:
- > data(energy)
- > attach(energy)
- > energy

Does energy expenditure differ between lean and obese women?

> t.test(expend ~ stature)

Welch Two Sample t-test 🗲

```
data: expend by stature
t = -3.8555, df = 15.919, p-value = 0.001411
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-3.459167 -1.004081
sample estimates:
mean in group lean mean in group obese
8.066154 10.297778
```

The same test, but group variances are assumed to be equal

> t.test(expend ~ stature, var.equal = TRUE)

Two Sample t-test

```
data: expend by stature
t = -3.9456, df = 20, p-value = 0.000799
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-3.411451 -1.051796
sample estimates:
mean in group lean mean in group obese
8.066154 10.297778
```

5. Classical Statistical Tests for Oneand Two-Sample Cases

5.3. Comparison of two variances

F-test to compare two variances

- Although R assumes that the group variances differ and thus automatically performs Welch test, we still can formally check this assumption:
- > var.test(expend ~ stature)

F test to compare two variances

```
data: expend by stature
F = 0.7844, num df = 12, denom df = 8, p-value = 0.6797
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
    0.1867876 2.7547991
sample estimates:
ratio of variances
    0.784446
```

Important limitations of F-test

- Not robust against departures from normality
- Not applicable to paired data

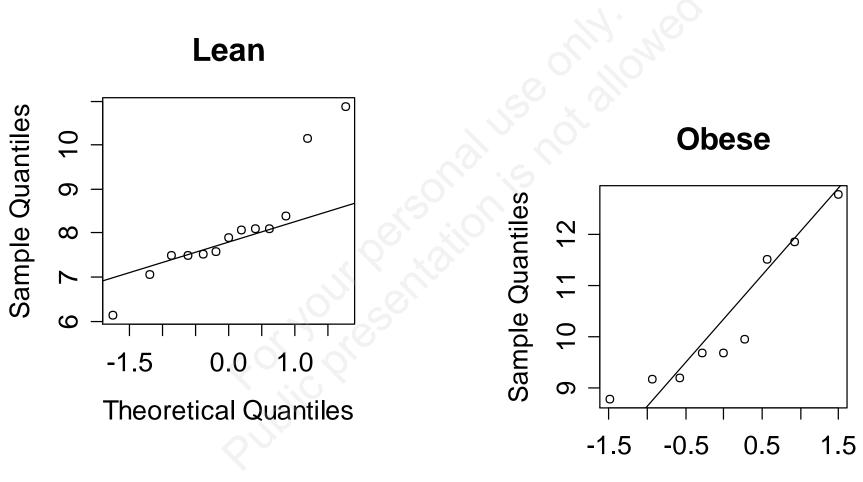
6. Classical Statistical Tests

6.4. Formal testing for normality

How to check if the data are normally distributed within each group?

- > e.lean <- energy\$expend[
 energy\$stature == "lean"]</pre>
- > e.obese <- energy\$expend[
 energy\$stature == "obese"]</pre>
- > qqnorm(e.lean); qqline(e.lean)
- > qqnorm(e.obese); qqline(e.obese)

Normal probability plots



Theoretical Quantiles

Shapiro-Wilk normality test

> shapiro.test(e.lean)

Shapiro-Wilk normality test

data: e.obese W = 0.876, p-value = 0.1426

No deviation from normality

Deviation from

shapiro.test(e.obese)

Shapiro-Wilk normality test normality (marginal) e.lean data: W = 0.8673, p-value = 0.04818

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6. Classical Statistical Tests for Oneand Two-Sample Cases

6.5. Wilcoxon test

Wilcoxon test

- t-tests are rather robust to deviations from normality, especially when samples are large
- However, in many cases (especially in Biology) it may be better to use a distribution-free method, e.g. Wilcoxon signed rank test

One-sample Wilcoxon test: theory

- H_0 : the sample of interest comes from a symmetric distribution with the center at μ_0
- Subtract theoretical μ_0 from each observation
- Rank the resultant values, ignoring the sign
- Sum up the positive ranks to get the V-statistic
- Compare V_{obs.} with V_{crit.}, or equivalently
- Calculate the probability P of obtaining $V \ge V_{obs.}$

An example...

Daily energy intake in 11 women compared against a recommended value:

> wilcox.test(d.intake, mu = 7725)

Wilcoxon signed rank test with continuity correction

```
data: d.intake
V = 8, p-value = 0.0293
alternative hypothesis: true location is not equal to 7725
```

```
Warning message:
In wilcox.test.default(d.intake, mu = 7725) :
    cannot compute exact p-value with ties
```

Two-sample Wilcoxon (= Mann Whitney) test: theory

- H₀: the two samples are coming from two distributions with different centers
- Rank all the values, ignoring the group and sign
- Sum up all the ranks in the first group to get the W-statistic
- Compare W_{obs} with its theoretically expected minimal value, or equivalently
- Or calculate the probability P of obtaining W ≥ W_{obs.}

An example...

- # Daily energy expenditure in lean and obese women
- > wilcox.test(expend ~ stature)

Wilcoxon rank sum test with continuity correction

data: expend by stature W = 12, p-value = 0.002122 alternative hypothesis: true location shift is not equal to 0

Limitation of the Wilcoxon tests

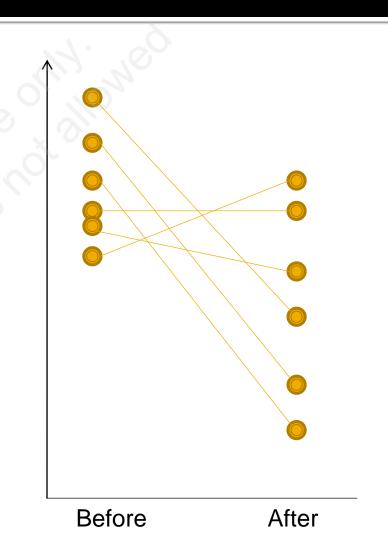
- Unless the sample size is 6 or above, the test simply cannot become significant at the 5% level
- The Wilcoxon tests are also susceptible to ties (i.e. observations sharing the same value). If ties are present, no exact *P*-value can be computed (not a problem for large samples)

6. Classical Statistical Tests for Oneand Two-Sample Cases

6.6. Comparison of paired samples

Paired tests

Paired tests are used when there are two measurements on the same experimental unit Individual differences are calculated and then averaged, reducing the problem to a onesample test



An example...

- > library(ISwR)
- # Pre- and postmenstrual energy intake in a group of women
- > data(intake)
- > intake
 - pre post
- 1 5260 3910
- 2 5470 4220
- 3 5640 3885

The same 11 women were measured, so it makes sense to look at individual differences:

> attach(intake)

> post - pre
[1] -1350 -1250 -1755 -1020 -745
[6] -1835 -1540 -1540 -725 -1330
[11] -1435

> mean(post-pre)
[1] -1320.455

Implementation of the paired t-test in R

Testing Ho that the mean individual difference equals to o:

> t.test(pre, post, paired = T)

```
Paired t-test
```

The matched-pairs Wilcoxon test

> wilcox.test(pre, post, paired = T)

Wilcoxon signed rank test with continuity correction

```
data: pre and post
V = 66, p-value = 0.00384
alternative hypothesis: true location shift is not equal to 0
```

```
Warning message:
In wilcox.test.default(pre, post, paired = T) :
    cannot compute exact p-value with ties
```

6. Classical Statistical Tests for Oneand Two-Sample Cases

6.7. Testing proportions

Testing a single proportion: the null hypothesis

 H_0 : the sample of interest comes from a population with the mean p_0

i.e. $H_0: p = p_0$

Testing a single proportion: the theory

The test is based on the binomial distribution
If *p* is the probability (proportion) of interest and *N* is the sample size, then

sample mean = Np

sample variance = Np(1-p)

If x is the number of binomial "successes", then the hypothesis of p = p₀ can be based on

$$u = \frac{x - Np_0}{\sqrt{Np_0(1 - p_0)}}$$

 u^2 has an approximate χ^2 distribution with 1 d.f.

An example...

- Suppose, 40 out of 250 (16%) randomly chosen snails were found to be infected with a parasite
- We want to test the hypothesis that the probability of a randomly selected mollusc being infected is the expected 0.20 (20%)



Implementation of the single proportion test in R

```
> prop.test(40, 250, 0.20)
```

1-sample proportions test with continuity correction

```
data: 40 out of 250, null probability 0.2
X-squared = 2.2563, df = 1, p-value = 0.1331
alternative hypothesis: true p is not equal to 0.2
95 percent confidence interval:
0.1180086 0.2127286
sample estimates:
    p
0.16
```

Comparison of two independent proportions

- Suppose, we examined two populations of snails
- In population 1, 24 out of 158 (15.2%) snails were infected
- In population 2, 15 out of 165 (9.1%) snails were infected
- Are the two proportions significantly different between populations?

Comparing two (or more) proportions in R

- # Data need to be presented in two vectors:
- # one with numbers of "successes"
 > infected <- c(24, 15)</pre>
- # and one with total numbers of observations:
 > examined <- c(158, 165)</pre>

Comparing two proportions in R

> prop.test(infected, examined)

2-sample test for equality of proportions with continuity correction

```
data: infected out of examined
x-squared = 2.2826, df = 1, p-value = 0.1308
alternative hypothesis: two.sided
95 percent confidence interval:
-0.01631243 0.13829171
sample estimates:
    prop 1     prop 2
0.15189873 0.09090909
```

The chi-squared test

Two (or more proportions) can also be compared with the function of the chi-squared test: chisq.test()

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

The data have to be presented as a matrix corresponding to a 2x2 contingency table

Comparing two proportions with the function chisq.test()

- > inf.data <- matrix(</pre>
 - c(infected, examined-infected),
 nrow = 2,
 - dimnames = list(c("Pop1", "Pop2"), c("Infected", "Noninfected")))

Comparing two proportions with the function chisq.test()

> chisq.test(inf.data)

Pearson's Chi-squared test with Yates' continuity correction

data: inf.data X-squared = 2.2826, df = 1, p-value = 0.1308

Fisher's exact test

- When the expected values are ≤5, the chisquared test produces unreliable P-values
- The Fisher's exact test has to be performed in such cases
- Suppose, the data on infection in snails were as follows:
- > infected <- c(1, 9)
- > examined <- c(9, 14)

Checking the expected values from a chi-squared test

> inf.data <- matrix(c(infected, examined-infected), nrow = 2, dimnames = list(c("Pop1", "Pop2"), c("Infected", "Noninfected"))) > chisq.test(inf.data)\$expected

Infected Noninfected
Pop1 3.913043 5.086957
Pop2 6.086957 7.913043
Warning message:
In chisq.test(inf.data) : Chi-squared approximation may be incorrect

Implementation of the Fisher's exact test in R

> fisher.test(inf.data)

Fisher's Exact Test for Count Data

data: inf.data p-value = 0.02881 alternative hypothesis: true odds ratio is not equal to 1 95 percent confidence interval: 0.001419354 0.867426585 sample estimates: odds ratio 0.07882877 # Compare with the chi-squared test's P-value: > chisq.test(inf.data) \$p.value

[1] 0.0376

6. Classical Statistical Tests for Oneand Two-Sample Cases

6.8. Extension of the chi-squared and Fisher's exact tests to *r* × *c* contingency tables

r × *c* contingency tables

- In many research setups, there can be >2 classes on both sides of a contingency table
- Suppose, we examined <u>3 populations</u> of snails
 In each population, we recorded the shell color
- ("light", "darker", "dark")
- Do the proportions of snails with various shell coloration differ among the three populations?

A 3 × 3 contingency table

- > light <- c(12, 40, 45)
- > darker <- c(87, 34, 75)
- > dark <- c(3, 8, 2)
- > color.data <- matrix(
 c(light, darker, dark), nrow = 3,
 dimnames = list(c(
 "Pop1", "Pop2", "Pop3"),</pre>
 - c("Light", "Darker", "Dark")))

A 3 × 3 contingency table

> color.data Light Darker Dark Pop1 12 87 3 Pop2 40 34 8 Pop3 45 75 2

Chi-squared test for a 3 x 3 table

> chisq.test(color.data)

```
Pearson's Chi-squared test
```

```
data: color.data 
X-squared = 43.4337, df = 4, p-value = 8.411e-09
```

```
Warning message:
In chisq.test(color.data) : Chi-squared approximation may be incorrect
```

Finding where the differences lie (i.e. influential cells)

- > E <- chisq.test(
 color.data)\$expected</pre>
- > 0 <- chisq.test(
 color.data)\$observed</pre>

 $> (O - E)^{2}/E$

Light Darker Dark Pop1 12.786942 7.1853741 0.4102564 Pop2 7.547399 6.5323333 5.8551423 Pop3 1.035041 0.1264774 1.9547594

Fisher's exact test for a 3 x 3 table

> fisher.test(color.data)

Fisher's Exact Test for Count Data

data: color.data
p-value = 1.191e-09
alternative hypothesis: two.sided