

R Lab #9b - Paired t -test

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Paired t -test

When you have two samples of data from a study with a *paired* design that means that both treatments are applied to every sampling unit. For these cases, a paired t -test should be used to test for equal means between the two groups, which has greater power compared to a standard two-sample t -test. Assumptions of the paired t -test include random sampling and that the distribution of differences in samples is normally distributed.

Chap12, Problem #11: ostrich temperatures

Problem #11 at the end of the chapter summarizes a study in which the researchers investigated if ostriches, which live in hot environments, have adaptations to cool their brain temperature compared to the rest of their body. The researchers measured both brain and body temperature in six individual ostriches (low sample size, but not trivial to catch an ostrich!). Since each ostrich had both “treatments” (i.e., brain and body temperature) this is a paired design.

Import data, calculate differences in samples

First, we should import the data and look at the data structure:

```
tempData <- read.csv("chap12q11OstrichTemp.csv", stringsAsFactors = T)
str(tempData)
## 'data.frame':    6 obs. of  3 variables:
## $ ostrich : int  1 2 3 4 5 6
## $ bodyTemp : num  38.5 38.5 38.3 38.5 38.6 ...
## $ brainTemp : num  39.3 39.2 39.2 38.7 39.1 ...
```

We can see that there are six rows (one for each ostrich) and three columns. The temperatures are stored in bodyTemp and brainTemp.

Since this is a paired design, we should calculate the difference in temperatures for each individual. The null hypothesis will then be that the distribution of differences has a mean of zero.

To add a new column to the data frame we simply establish a new variable and set that as the difference between bodyTemp and brainTemp:

```
tempData$diff <- tempData$bodyTemp - tempData$brainTemp
str(tempData)
## 'data.frame':   6 obs. of  4 variables:
## $ ostrich : int  1 2 3 4 5 6
## $ bodyTemp : num  38.5 38.5 38.3 38.5 38.6 ...
## $ brainTemp: num  39.3 39.2 39.2 38.7 39.1 ...
## $ diff : num  -0.81 -0.76 -0.93 -0.16 -0.47 ...
```

Now we have a sample of six differences.

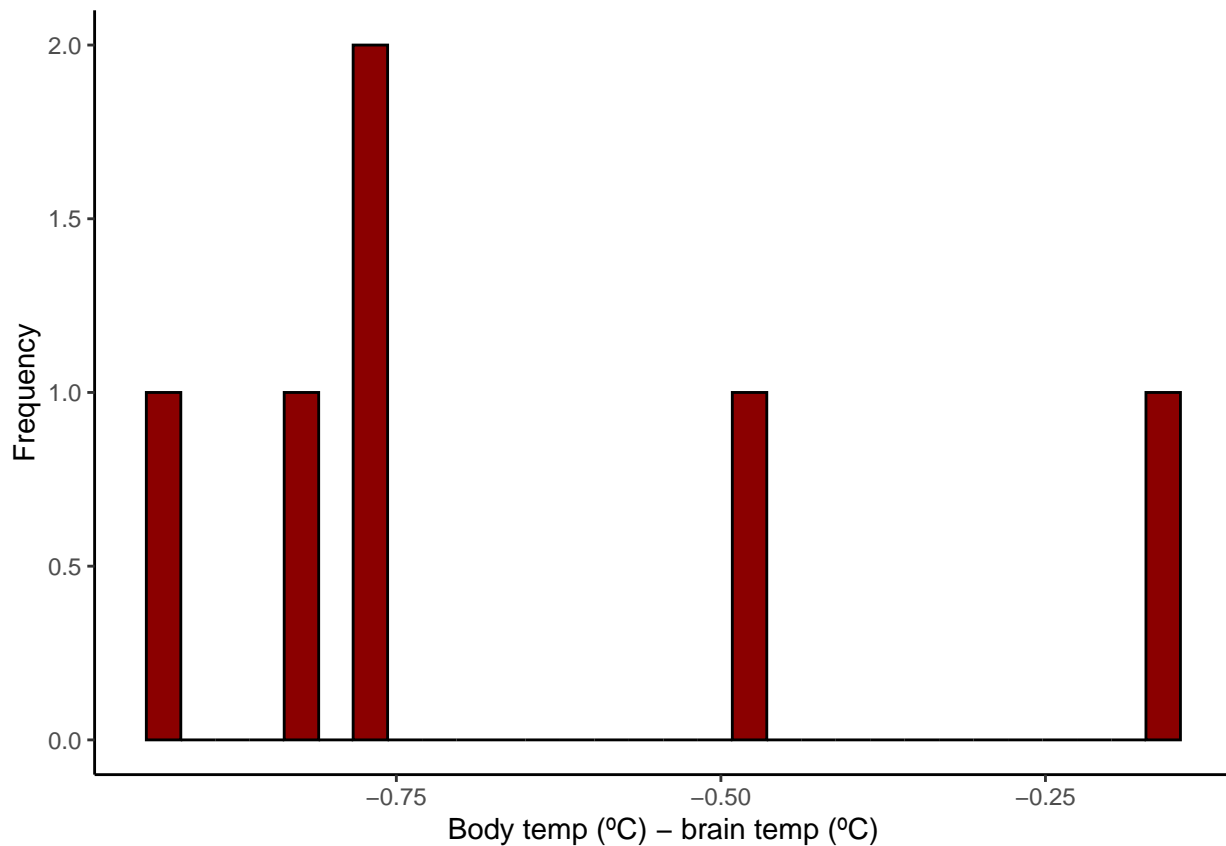
Using graphs to evaluate normality

An assumption of the paired t -test is that the sample of differences has a normal distribution.

Generally, it is useful to graph the data to see if it appears normal. However, with $n=6$ in this case plots will not be very informative.

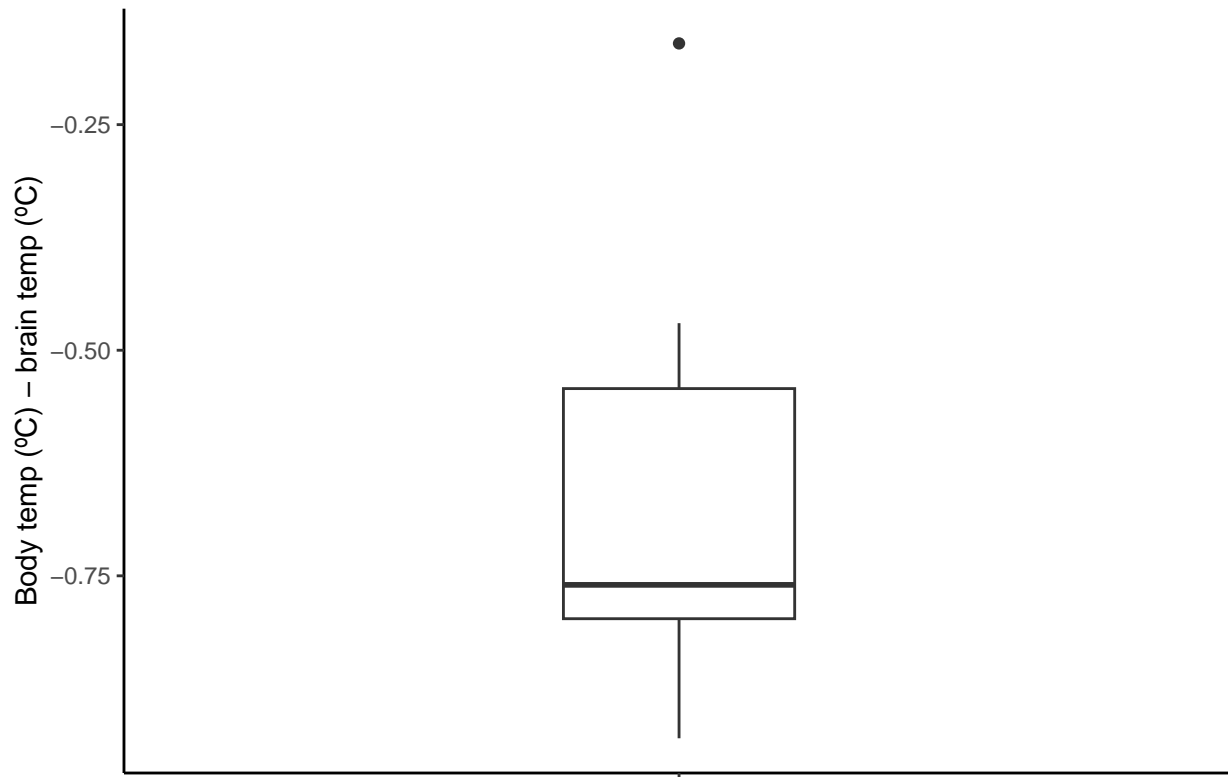
Nonetheless, let's work through some possible plots that you could make to qualitatively look for normality. First, let's make a frequency distribution histogram:

```
library(ggplot2)
ggplot(tempData, aes(x = diff)) + geom_histogram(color = "black",
  fill = "darkred") + xlab("Body temp (°C) - brain temp (°C)") +
  ylab("Frequency") + theme_classic()
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



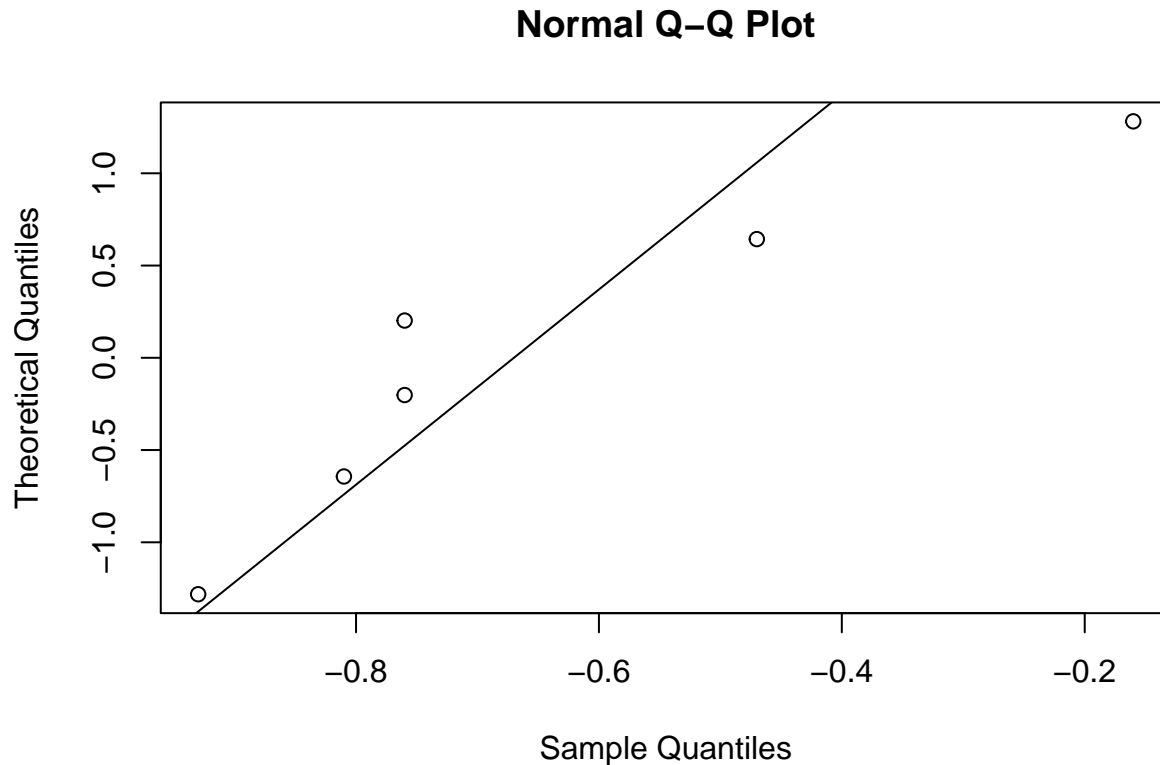
Now a box plot for a different perspective:

```
ggplot(tempData, aes(x = "", y = diff)) + geom_boxplot(width = 0.25) +
  xlab("") + ylab("Body temp (°C) - brain temp (°C)") + theme_classic()
```



Now a normal quantile plot:

```
qqnorm(tempData$diff, datax = T)
qqline(tempData$diff, datax = T)
```



All of these plots suggest that there is some right skew, but again the sample size is low so the potential for the data appearing non-normal (even if the population distribution is normal) is high.

Formally testing for normality

There are also tests that quantitatively test if a sample is consistent with a normal distribution, and the most common is the Shapiro-Wilk test (see Chap 13). This test calculates the mean and standard deviation of your sample, uses these estimates to derive a corresponding normal distribution, and then does a goodness-of-fit between the two.

The command to run the test is `shapiro.test(x)`, where `x` is a vector of numbers. The null hypothesis is that the sample has a normal distribution.

```
shapiro.test(tempData$diff)
##
##  Shapiro-Wilk normality test
##
## data:  tempData$diff
## W = 0.87033, p-value = 0.2275
```

The P -value of the test is above 0.05, and thus we fail to reject the null hypothesis that our sample of differences has a normal distribution.

Running the paired t -test

Since we failed to reject that our sample of differences has a normal distribution we can proceed to running a paired t -test.

There are two ways that we can run this test in R. One way is to now effectively treat it as a one-sample t -test with the sample of differences as input and a null μ of zero.

```
t.test(tempData$diff, mu = 0)
##
## One Sample t-test
##
## data: tempData$diff
## t = -5.6099, df = 5, p-value = 0.002489
## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
## -0.9454120 -0.3512547
## sample estimates:
## mean of x
## -0.6483333
```

Another way is to run a *t*-test with two samples as input (bodyTemp and brainTemp) and add the argument that the samples are paired:

```
t.test(tempData$bodyTemp, tempData$brainTemp, paired = T)
##
## Paired t-test
##
## data: tempData$bodyTemp and tempData$brainTemp
## t = -5.6099, df = 5, p-value = 0.002489
## alternative hypothesis: true mean difference is not equal to 0
## 95 percent confidence interval:
## -0.9454120 -0.3512547
## sample estimates:
## mean difference
## -0.6483333
```

Notice that both of these commands produce the same exact output. The *P*-value of the test is 0.002489, which is below an alpha of 0.05. Thus, we reject the null hypothesis of the sample of differences having a mean of zero or the two samples having the same mean.

How do we interpret this biologically? The order of variables is important. We can see in both tests that the 95% CI is negative and does not contain zero.

In the one-sample command we input the differences, and when those were calculated we did bodyTemp-brainTemp. Thus, the negative 95% CI of the mean being negative means that the second sample (brainTemp) is higher.

Similarly, in the two-sample command we gave bodyTemp as the first argument and brainTemp as the second argument. By default R subtracts the second sample/argument from the first.

If we re-run the two-sample command with the opposite order of variables then the *t*-statistic will be the same number with a positive sign, the *P*-value will be the same, and the 95% CI of the difference will also be the same but with a positive sign.

```
t.test(tempData$brainTemp, tempData$bodyTemp, paired = T)
##
## Paired t-test
##
## data: tempData$brainTemp and tempData$bodyTemp
## t = 5.6099, df = 5, p-value = 0.002489
## alternative hypothesis: true mean difference is not equal to 0
## 95 percent confidence interval:
## 0.3512547 0.9454120
## sample estimates:
```

```
## mean difference  
## 0.648333
```

R commands summary

- **Paired *t*-test**
 - `t.test(differences,mu=0)` OR `t.test(sample1,sample2,paired=T)`
- **Shapiro-Wilk test**
 - `shapiro.test(x)`