

Multivariate Statistics in Ecology and Quantitative Genetics

Hotellings T^2

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http://evol.bio.lmu.de/_statgen

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- 3 Multivariate normal distribution
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- 5 Hotellings T^2 -test
- 6 Testing for normality

We want to compare the vegetative growth of a mutated raspberry (*Rubus idaeus*) with the wildtype raspberry.



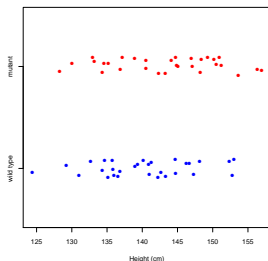
We want to compare the vegetative growth of a mutated raspberry (*Rubus idaeus*) with the wildtype raspberry.



We use different quantities as a measure for “growth”:

- Height of bush (cm)
- Width of bush (cm)

Comparison of height:

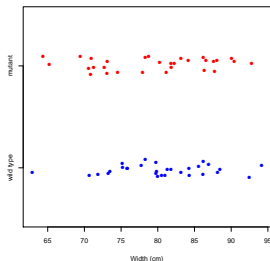


```
> raspberry <- read.table("raspberry.csv",sep="," ,h=T)
> attach(raspberry)
> height.wt <- height[type=="wild type"]
> height.mu <- height[type=="mutant"]
> t.test(height.wt,height.mu)
```

Welch Two Sample t-test

```
data: height.wt and height.mu
t = -1.5441, df = 57.334, p-value = 0.1281
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -6.7627773  0.8735087
sample estimates:
mean of x mean of y
 140.2768  143.2215
```

Comparison of width:



```
> width.wt <- width [type=="wild type"]
> width.mu <- width [type=="mutant"]
> t.test(width.wt,width.mu)
```

Welch Two Sample t-test

```
data: width.wt and width.mu
t = 0.5717, df = 56.645, p-value = 0.5698
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.709472  4.874489
sample estimates:
mean of x mean of y
 80.47013  79.38762
```

Both height and width are not significantly different for the the types of raspberry.

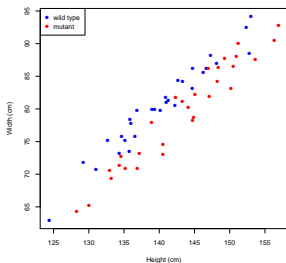
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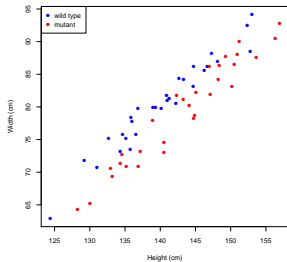
Is there no difference?

Both height and width are not significantly different for the the types of raspberry.

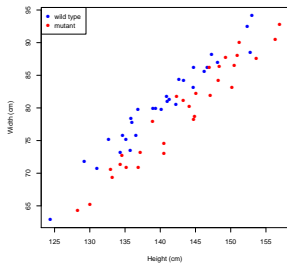
Is there no difference?

So far, we have not exploited the correlation between height and width. Let's look at the bivariate (two variables) data:





We see a difference “by eye”. Which test can detect that?



We see a difference “by eye”. Which test can detect that?
Answer: We need the multivariate analogon of the t-test. This multivariate version of the t-test is called

Hotelling's T^2 -test

(Before we come to this test, we need to learn some theory)

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- An d -dimensional random vector is a vector of d random elements
- The expectation of a random vector $X = (X_1, X_2, \dots, X_d)^T$ is the vector of the expectations:

$$\mathbb{E}X = \mathbb{E} \begin{pmatrix} X_1 \\ \vdots \\ X_d \end{pmatrix} = \begin{pmatrix} \mathbb{E}X_1 \\ \vdots \\ \mathbb{E}X_d \end{pmatrix}$$

- The vector (height,width) is a two-dimensional random vector (with values in \mathbb{R}^2)
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- The expectation of a random matrix $M = (M_{ij})_{i=1..n,j=1..d}$ is the matrix of the expectations:

$$\mathbb{E} \begin{pmatrix} M_{11} & M_{12} & \cdots & M_{1d} \\ \vdots & & \ddots & \vdots \\ M_{n1} & M_{n2} & \cdots & M_{nd} \end{pmatrix} = \begin{pmatrix} \mathbb{E}M_{11} & \mathbb{E}M_{12} & \cdots & \mathbb{E}M_{1d} \\ \vdots & \ddots & & \vdots \\ \mathbb{E}M_{n1} & \mathbb{E}M_{n2} & \cdots & \mathbb{E}M_{nd} \end{pmatrix}$$

- reminder: The variance of a univariate random variable X is
$$\text{Var}(X) = \mathbb{E} [(X - \mathbb{E}X)^2] = \mathbb{E} [X^2] - (\mathbb{E}X)^2.$$

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- The analog in the multivariate case is the so called *covariance matrix* (or dispersion matrix or variance-covariance matrix). The covariance matrix $\text{Var}(X) = \Sigma$ of $X = (X_1, \dots, X_d)^T$ is

$$\begin{aligned} \Sigma &= \begin{pmatrix} \text{Cov}(X_1, X_1) & \text{Cov}(X_1, X_2) & \cdots & \text{Cov}(X_1, X_d) \\ \text{Cov}(X_2, X_1) & \text{Cov}(X_2, X_2) & \cdots & \text{Cov}(X_2, X_d) \\ \vdots & & \ddots & \vdots \\ \text{Cov}(X_d, X_1) & \text{Cov}(X_d, X_2) & \cdots & \text{Cov}(X_d, X_d) \end{pmatrix} \\ &= \mathbb{E} \left[\begin{pmatrix} X_1 - \mathbb{E}X_1 \\ \vdots \\ X_d - \mathbb{E}X_d \end{pmatrix} \cdot (X_1 - \mathbb{E}X_1, \dots, X_d - \mathbb{E}X_d) \right] \\ &= \mathbb{E} \left[(X - \mathbb{E}X) \cdot (X - \mathbb{E}X)^T \right] \\ &= \mathbb{E} \left[X \cdot X^T \right] - \mathbb{E}X \cdot (\mathbb{E}X)^T \end{aligned}$$

- Linearity of the expectation is analogous to the univariate case: Let $X = (X_1, \dots, X_d)$ be a random vector and $C = (C_{ij})_{i=1..n, j=1..d}$ be a deterministic matrix. Then

$$\mathbb{E}(C \cdot X) = C \cdot \mathbb{E}(X)$$

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$$\mathbb{E}(C \cdot X) = C \cdot \mathbb{E}(X)$$

- If $Y := X - \mathbb{E}(X)$, then

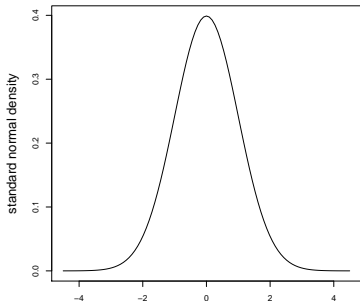
$$\begin{aligned} \text{Var}(C \cdot X) &= \text{Var}(C \cdot Y) \\ &= \mathbb{E} [C \cdot Y \cdot (C \cdot Y)^T] \\ &= \mathbb{E} [C \cdot Y \cdot Y^T \cdot C^T] \\ &= C \cdot \mathbb{E} [Y \cdot Y^T] \cdot C^T \\ &= C \cdot \text{Var}(Y) \cdot C^T \\ &= C \cdot \text{Var}(X) \cdot C^T \end{aligned}$$

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- **Reminder:** Univariate normal distribution $\mathcal{N}(\mu, \sigma^2)$ with mean $\mu \in \mathbb{R}$ and variance $\sigma^2 \in (0, \infty)$ has the density

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x - \mu)^2}{2\sigma^2}\right)$$



Remember: $\mathbb{P}(\mu - \sigma < X < \mu + \sigma) = 0.68$ and
 $\mathbb{P}(\mu - 1.96\sigma < X < \mu + 1.96\sigma) = 0.95$

- The density of the d -dimensional normal distribution with mean $\mu \in \mathbb{R}^d$ and covariance matrix $\Sigma \in \mathbb{R}^{d \times d}$ is analogous:

$$f(x) = \frac{1}{\sqrt{(2\pi)^d \det(\Sigma)}} \exp\left(-\frac{(x - \mu)^T \Sigma^{-1} (x - \mu)}{2}\right)$$

for $x \in \mathbb{R}^d$ where $\det(\Sigma)$ is the determinand of Σ , and Σ^{-1} is the inverse matrix.

We write $\mathcal{N}_d(\mu, \Sigma)$ for this distribution.

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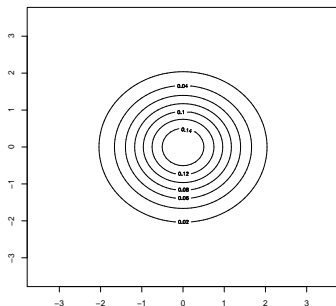
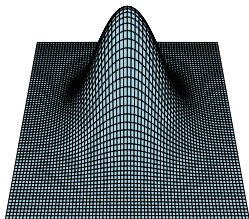
We write $\mathcal{N}_d(\mu, \Sigma)$ for this distribution.

- The *standard multivariate normal distribution* has mean $\mu = 0$ and the identity matrix $\Sigma = \mathbb{1}$ as covariance matrix.

Plots for $d = 2$

Correlation 0.0:

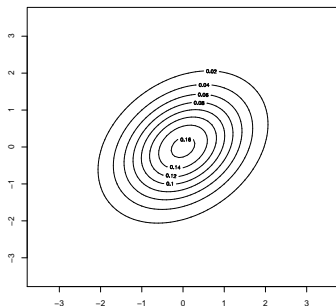
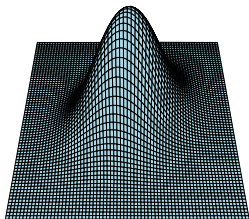
$$\Sigma = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, \text{Var}(X_1) = 1 = \text{Var}(X_2), \text{Cov}(X_1, X_2) = 0.0$$



Plots for $d = 2$

Correlation 0.3:

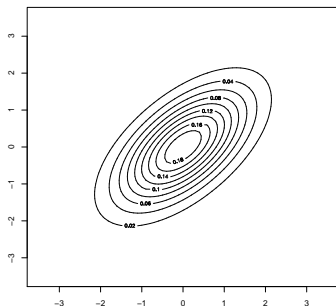
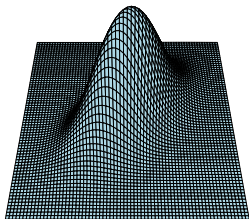
$$\Sigma = \begin{pmatrix} 1 & 0.3 \\ 0.3 & 1 \end{pmatrix}, \text{Var}(X_1) = 1 = \text{Var}(X_2), \text{Cov}(X_1, X_2) = 0.3$$



Plots for $d = 2$

Correlation 0.6:

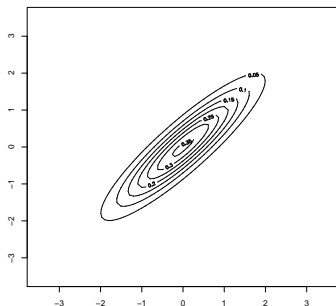
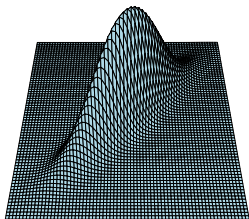
$$\Sigma = \begin{pmatrix} 1 & 0.6 \\ 0.6 & 1 \end{pmatrix}, \text{Var}(X_1) = 1 = \text{Var}(X_2), \text{Cov}(X_1, X_2) = 0.6$$



Plots for $d = 2$

Correlation 0.9:

$$\Sigma = \begin{pmatrix} 1 & 0.9 \\ 0.9 & 1 \end{pmatrix}, \text{Var}(X_1) = 1 = \text{Var}(X_2), \text{Cov}(X_1, X_2) = 0.9$$



Properties: (Let the distribution of X be $\mathcal{N}_d(\mu, \Sigma)$)

- Linear combinations are univariate normal distributed:
 $\langle \mathbf{c}, \mathbf{X} \rangle \sim \mathcal{N}(\langle \mathbf{c}, \mu \rangle, \mathbf{c}\Sigma\mathbf{c}^T)$

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- X_i and X_j are independent $\iff \text{Cov}(X_i, X_j) = 0$
- The standardized normal distribution is standard normal distributed

$$\Sigma^{-\frac{1}{2}} \cdot (X - \mu) \sim \mathcal{N}_d(\mathbf{0}, \mathbb{1})$$

where $M = \Sigma^{-\frac{1}{2}}$ is a matrix such that $M^T \cdot M \cdot \Sigma = \mathbb{1}$.

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- The square of the standardized normal distribution is chi-squared distributed with d degrees of freedom:

$$(X - \mu)^T \Sigma^{-1} (X - \mu) \sim \chi_d^2.$$

Properties: (Let the distribution of X be $\mathcal{N}_d(\mu, \Sigma)$)

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- The square of the standardized normal distribution is chi-squared distributed with d degrees of freedom:

$$(X - \mu)^T \Sigma^{-1} (X - \mu) \sim \chi_d^2.$$

- If Y_1, Y_2, \dots, Y_d are independent and standard normal distributed, then $(Y_1, \dots, Y_d) \sim \mathcal{N}(\mathbf{0}, \mathbb{1})$.

Properties: (Let the distribution of X be $\mathcal{N}_d(\mu, \Sigma)$)

- If $M \in \mathbb{R}^{p \times d}$ is a non-random matrix, then
 $M \cdot X \sim \mathcal{N}_p(M \cdot \mu, M \Sigma M^T)$

Estimating μ and Σ

Let Y_1, \dots, Y_n be a sample of independent observations from a $\mathcal{N}_d(\mu, \Sigma)$ distribution. As usual, we write the variables as columns and the different observations as rows:

$$Y = \begin{pmatrix} Y_{11} & Y_{12} & \cdots & Y_{1d} \\ Y_{21} & Y_{22} & \cdots & Y_{2d} \\ \vdots & & \ddots & \vdots \\ Y_{n1} & Y_{n2} & \cdots & Y_{nd} \end{pmatrix}$$

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Estimating μ and Σ

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- The sample mean \bar{Y} where $\bar{Y}_j := \frac{1}{n} \sum_{i=1}^n Y_{ij}$ is an estimator for μ .
- The sample covariance matrix $S := (S_{ij})_{i,j=1..d}$ where

$$S_{ij} = \frac{1}{n-1} \sum_{k=1}^n (Y_{ki} - \bar{Y}_i) (Y_{kj} - \bar{Y}_j)$$

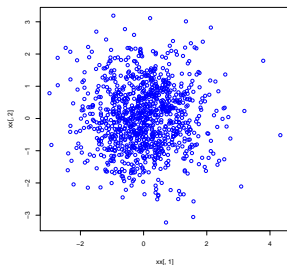
is an (unbiased) estimator for Σ .

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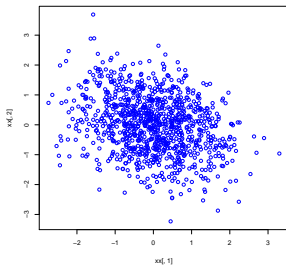
```
> library("mvtnorm") # multivariate t- and normal distrib.  
> Sigma0 <- matrix(c(1,0,0,1),ncol=2)  
> dmvnorm(c(0,0),mean=c(0,0),sigma=Sigma0)  
[1] 0.1591549  
> dmvnorm(c(0,0)) # same, the standard normal is the default  
[1] 0.1591549
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> dmvnorm(c(0,0)) # same, the standard normal is the default  
[1] 0.1591549  
  
> xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma0)  
> plot(xx[,1],xx[,2])
```



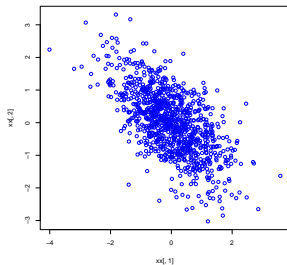
Correlation -0.3 :

```
> Sigma3 <- matrix(c(1,-0.3,-0.3,1),ncol=2)
> xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma3)
> plot(xx[,1],xx[,2])
```



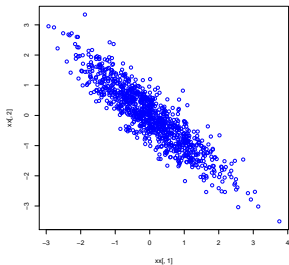
Correlation -0.6 :

```
> Sigma6 <- matrix(c(1,-0.6,-0.6,1),ncol=2)
> xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma6)
> plot(xx[,1],xx[,2])
```



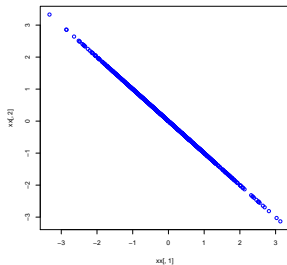
Correlation -0.9 :

```
> Sigma9 <- matrix(c(1,-0.9,-0.9,1),ncol=2)
> xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma9)
> plot(xx[,1],xx[,2])
```



Correlation -1:

```
> Sigma10 <- matrix(c(1,-1,-1,1),ncol=2)
> xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma10)
> plot(xx[,1],xx[,2])
```



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- Reminder for univariate case $d = 1$: If $X = (X_1, \dots, X_n)$ is an independent sample from the $\mathcal{N}_1(\mu, \sigma^2)$ distribution, then the t -statistics

$$t := \frac{\bar{X} - \mu}{sd(X)/\sqrt{n}}$$

is t -distributed with $n - 1$ degrees of freedom. This fact is used for the t -test.

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$$t := \frac{\bar{X} - \mu}{sd(X)/\sqrt{n}}$$

is t -distributed with $n - 1$ degrees of freedom. This fact is used for the t-test. In addition, $t^2 \sim \mathcal{F}_{1, n-1}$ is Fisher-distributed with 1 numerator degree of freedom and $n - 1$ denominator degrees of freedom.

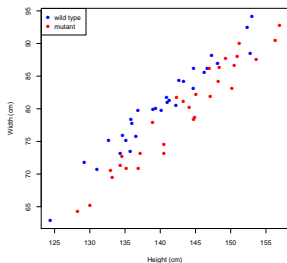
- Multivariate case: If $X := (X_1, \dots, X_n)$ be a sample from the $\mathcal{N}_d(\mu, \Sigma)$ distribution with $n > d$, then the statistics

$$T^2 := n (\bar{X} - \mu)^T S^{-1} (\bar{X} - \mu)$$

is called *Hotellings T^2* . The matrix S^{-1} is the inverse of the sample covariance matrix. It has been shown that

$$T^2 \sim \frac{(n-1)d}{n-d} \mathcal{F}_{d, n-d}$$

Back to our raspberry example:



We see a difference “by eye”. Does Hotellings T^2 -test detect this difference?


```
> raspberry <- read.table("raspberry.csv",sep=",",header=T)
> raspberry.wt<-subset(raspberry,type=="wild type",select=-3)
> raspberry.mu<-subset(raspberry,type=="mutant",select=-3)
> library(rrcov) #one out of many libraries with Hotelling T2
> T2.test(raspberry.wt,raspberry.mu)
```

Hotelling's two sample T^2 -test

```
data:  raspberry.wt and raspberry.mu
T.2 = 36.7558, df1 = 2, df2 = 57, p-value = 5.577e-11
alternative hypothesis: true location difference is not
equal to c(0,0)
sample estimates:
                height      width
mean x-vector 143.2215  79.38762
mean y-vector 140.2768  80.47013
```

Hotellings T^2 -test detects a significant difference

```
# alternative syntax with a formula  
> T2.test(cbind(height,width)~type,data=raspberry)
```

```
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> T2.test(cbind(height,width)~type,data=raspberry)
```

Syntax:

```
## Default S3 method:  
T2.test(x, y = NULL, mu = 0, conf.level = 0.95, ...)
```

```
## S3 method for class 'formula':  
T2.test(formula, data, subset, na.action, ...)
```

If $y = \text{NULL}$, then perform a one sample T^2 -test with null hypothesis H_0 : true mean is μ

Directions for use:

- The T^2 -test (like the t-test) is prone to outliers. A single outlier can considerably decrease the power of the test. So check for outliers (possible mismeasurements)

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- If one of the variables shows already a significant difference between the group, then also the T^2 -test yields a significant difference.

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A multivariate Shapiro-Wilk test for normality is

`mshapiro.test()` in the library `mvnormtest`:

```
# install.packages("mvnormtest") # install library if not installed
> library(mvnormtest)
> library(mvtnorm)
> x <- rmvnorm(100,mean=c(0,0))
> mshapiro.test(t(x))
```

Shapiro-Wilk normality test

data: Z

W = 0.9915, p-value = 0.7839