Multivariate Statistics in Ecology and Quantitative Genetics
Hotellings T^2

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

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Contents

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

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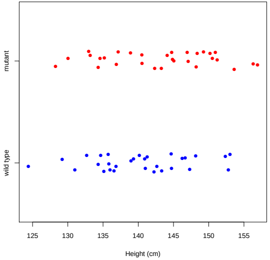
We use different quantities as a measure for “growth”:

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

Comparison of height:

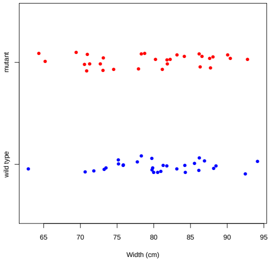



```
> raspberry <- read.table("raspberry.csv",sep=",",h=T)
> attach(raspberry)
> height.wt <- height[type=="wild type"]
> height.mu <- height[type=="mutant"]
> t.test(height.wt,height.mu)

        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?

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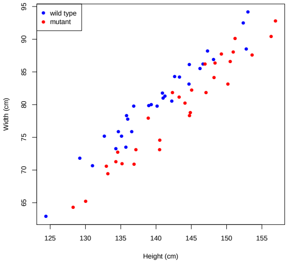
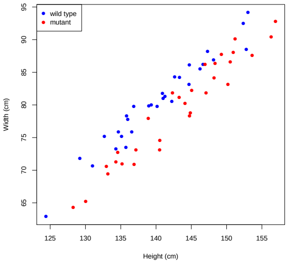
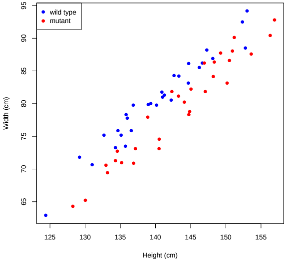
Is there no difference?

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)

Contents

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- The expectation of a random vector $X = (X_1, X_2, \ldots, X_d)^T$ is the vector of the expectations:

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

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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 $\text{Var}(X) = \mathbb{E}\left[(X - \mathbb{E}X)^2\right] = \mathbb{E}\left[X^2\right] - (\mathbb{E}X)^2$.

- reminder: The variance of a univariate random variable X is $\mathrm{Var}(X) = \mathbb{E}\left[(X - \mathbb{E}X)^2\right] = \mathbb{E}\left[X^2\right] - (\mathbb{E}X)^2$.
- The analog in the multivariate case is the so called *covariance matrix* (or dispersion matrix or variance-covariance matrix). The covariance matrix $\mathrm{Var}(X) = \Sigma$ of $X = (X_1, \ldots, X_d)^T$ is

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

- Linearity of the expectation is analogos to the univarite case: Let $X = (X_1, \ldots, X_d)$ 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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$$\mathbb{E}(C \cdot X) = C \cdot \mathbb{E}(X)$$

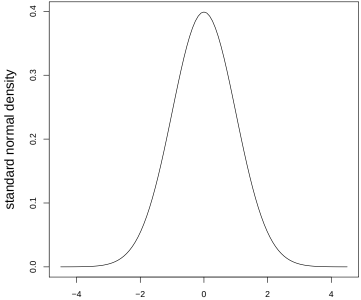
- If $Y := X - \mathbb{E}(X)$, then

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

Contents

- 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 $\mathcal{N}_d\left(\mu, \Sigma\right)$ for this distribution.

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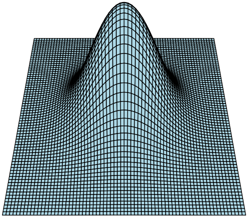
  for $x \in \mathbb{R}^d$ where $\det(\Sigma)$ is the determinand of Σ, and Σ^{-1} is the inverse matrix.
  We write $\mathcal{N}_d(\mu, \Sigma)$ for this distribution.

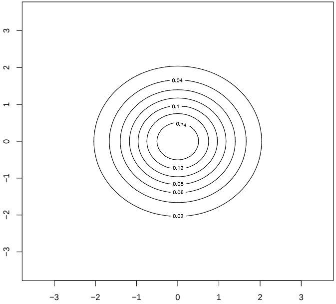
- The *standard multivariate normal distribution* has mean $\mu = 0$ and the identity matrix $\Sigma = \mathbb{1}$ as covariance matrix.

Plots for $d = 2$

Correlation 0.0:

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

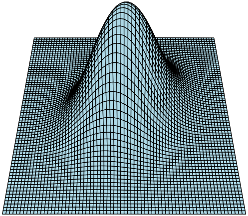


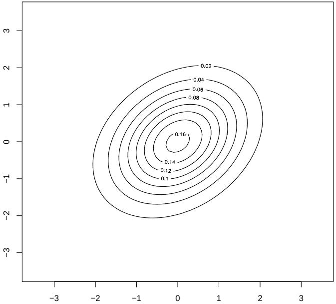


Plots for $d = 2$

Correlation 0.3:

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

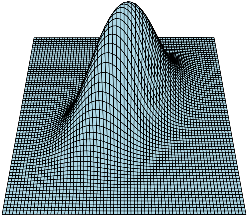


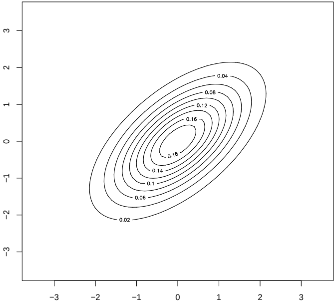


Plots for $d = 2$

Correlation 0.6:

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

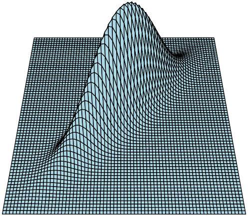


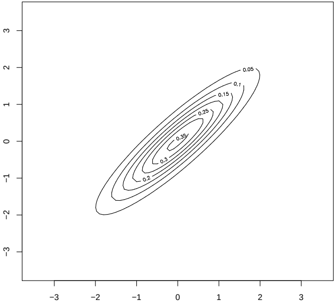


Plots for $d = 2$

Correlation 0.9:

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





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

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

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- X_i and X_j are independent $\iff$ $\mathrm{Cov}(X_i, X_j) = 0$
- The standardized normal distribution is standard normal distributed
$$\Sigma^{-\frac{1}{2}} \cdot (X - \mu) \sim \mathcal{N}_d(0, \mathbb{1})$$
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:
$$(X - \mu)^T \Sigma^{-1} (X - \mu) \sim \chi^2_d.$$
- If $Y_1, Y_2, \dots, Y_d$ are independent and standard normal distributed, then $(Y_1, \dots, Y_d) \sim \mathcal{N}(0, \mathbb{1})$.

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 $M \cdot X \sim \mathcal{N}_p\left(M \cdot \mu, M \Sigma M^T\right)$

Estimating μ and Σ

Let $Y_{1\cdot}, \ldots, Y_{n\cdot}$ 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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- 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

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

is an (unbiased) estimator for Σ.

Contents

```
> 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
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```

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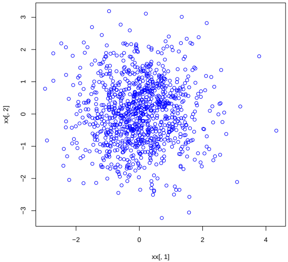
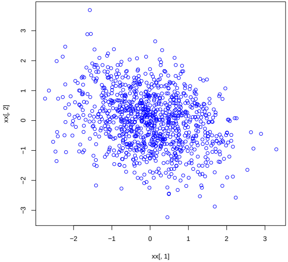
> xx  <- rmvnorm(1000,mean=c(0,0),sigma=Sigma0)
> plot(xx[,1],xx[,2])
```



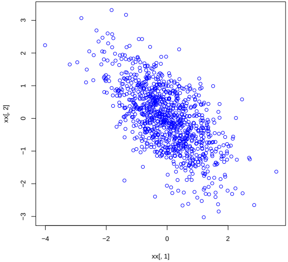
Correlation -0.3:

```
> 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])
```



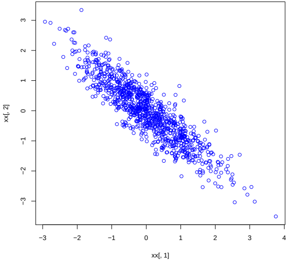
Correlation -0.6:

```
> 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])
```



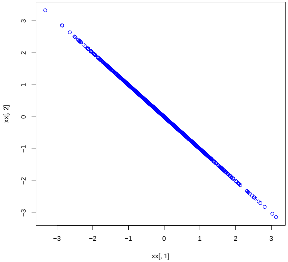
Correlation -0.9:

```
> 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])
```



Contents

- Reminder for univarite case $d = 1$: If $X = (X_1, \ldots, X_n)$ 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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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.

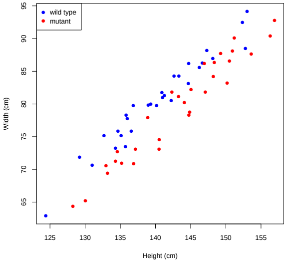
- Multivariate case: If $X := (X_{1,\cdot}, \dots, X_{n,\cdot}$ be a sample from the $\mathcal{N}_d(\mu, \Sigma)$ distribution with $n > d$, then the statistics

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

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 T2-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 = NULL`, then perform a one sample T^2-test with null hypothesis $H_0:$ true mean is `mu`

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.

Contents

A multivariate Shapiro-Wilk test for normality is `mshapiro.test()` in the library `mvnormtest`:

```
# install.packages("mvnormtest")  # install library if i
> 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
```