Multivariate Statistics in Ecology and Quantitative Genetics Hotellings T²

Dirk Metzler & Noémie Becker

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

Summer semester 2014



Contents

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in F
- 6 Hotellings T²-test
- 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:

140 2768 143 2215

```
25 130 135 140 145 150 155 Halpt (im)
```

> raspberry <- read.table("raspberry.csv",sep=",",h=T)

Comparison of width:

```
65 70 75 80 85 90 95 Width (mi)
```

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

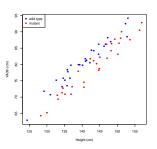
Both height and width are not significantly different for the types of raspberry.

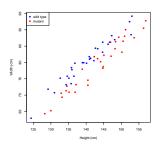
Is there no difference?

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

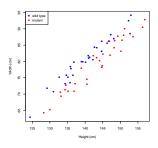
Is there no difference?

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 T2-test

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

Contents

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in F
- 6 Hotellings T²-test
- Testing for normality

• The vector (height,width) is a two-dimensional random vector (with values in \mathbb{R}^2)

- The vector (height,width) is a two-dimensional random vector (with values in \mathbb{R}^2)
- An d-dimensional random vector is a vector of d random elements

- The vector (height,width) is a two-dimensional random vector (with values in \mathbb{R}^2)
- 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)
- 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 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 $Var(X) = \mathbb{E}\left[(X - \mathbb{E}X)^2\right] = \mathbb{E}\left[X^2\right] - (\mathbb{E}X)^2$.

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

$$\Sigma = \begin{pmatrix} \operatorname{Cov}(X_1, X_1) & \operatorname{Cov}(X_1, X_2) & \cdots & \operatorname{Cov}(X_1, X_d) \\ \operatorname{Cov}(X_2, X_1) & \operatorname{Cov}(X_2, X_2) & \cdots & \operatorname{Cov}(X_2, X_d) \\ \vdots & & \ddots & \vdots \\ \operatorname{Cov}(X_d, X_1) & \operatorname{Cov}(X_d, X_2) & \cdots & \operatorname{Cov}(X_d, X_d) \end{pmatrix}$$

$$= \mathbb{E} \left[\begin{pmatrix} X_1 - \mathbb{E}X_1 \\ \vdots \\ X_d - \mathbb{E}X_d \end{pmatrix} \cdot \begin{pmatrix} X_1 - \mathbb{E}X_1, \cdots, X_d - \mathbb{E}X_d \end{pmatrix} \right]$$

$$= \mathbb{E} \left[(X - \mathbb{E}X) \cdot (X - \mathbb{E}X)^T \right]$$

$$= \mathbb{E} \left[X \cdot X^T \right] - \mathbb{E}X \cdot (\mathbb{E}X)^T$$

• 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

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

• 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

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

• If $Y := X - \mathbb{E}(X)$, then

$$Var(C \cdot X) = Var(C \cdot Y)$$

$$= \mathbb{E} \left[C \cdot Y \cdot (C \cdot Y)^T \right]$$

$$= \mathbb{E} \left[C \cdot Y \cdot Y^T \cdot C^T \right]$$

$$= C \cdot \mathbb{E} \left[Y \cdot Y^T \right] \cdot C^T$$

$$= C \cdot Var(Y) \cdot C^T$$

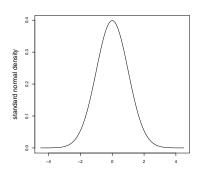
$$= C \cdot Var(X) \cdot C^T$$

Contents

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in F
- 6 Hotellings T²-test
- Testing for normality

• 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.

• 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:

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

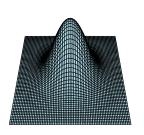
for $x \in \mathbb{R}^d$ where $\det(\Sigma)$ is the determinand of Σ , and Σ^{-1} is the inverse matrix.

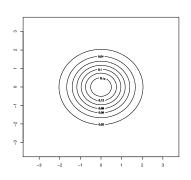
We write $\mathcal{N}_{d}(\mu, \Sigma)$ for this distribution.

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

Correlation 0.0:

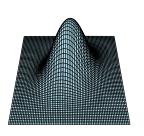
$$\Sigma = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$$
, $Var(X_1) = 1 = Var(X_2)$, $Cov(X_1, X_2) = 0.0$

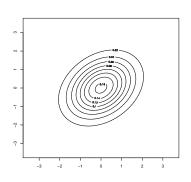




Correlation 0.3:

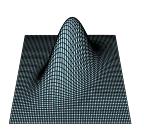
$$\Sigma = \begin{pmatrix} 1 & 0.3 \\ 0.3 & 1 \end{pmatrix}$$
, $Var(X_1) = 1 = Var(X_2)$, $Cov(X_1, X_2) = 0.3$

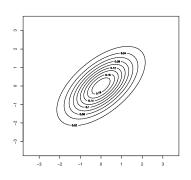




Correlation 0.6:

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

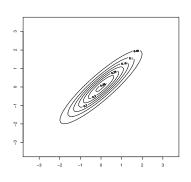




Correlation 0.9:

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





• Linear combinations are univariate normal distributed:

$$\langle \boldsymbol{c}, \boldsymbol{X} \rangle \sim \mathcal{N}\left(\langle \boldsymbol{c}, \boldsymbol{\mu} \rangle, \boldsymbol{c} \boldsymbol{\Sigma} \boldsymbol{c}^{\mathsf{T}}\right)$$

- Linear combinations are univariate normal distributed: $\langle c, X \rangle \sim \mathcal{N} \left(\langle c, \mu \rangle, c \Sigma c^T \right)$
- X_i and X_j are independent \iff $Cov(X_i, X_j) = 0$

- Linear combinations are univariate normal distributed: $\langle c, X \rangle \sim \mathcal{N} \left(\langle c, \mu \rangle, c \Sigma c^T \right)$
- X_i and X_j are independent \iff $Cov(X_i, X_j) = 0$
- The standardized normal distribution is standard normal distributed

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

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

- Linear combinations are univariate normal distributed: $\langle c, X \rangle \sim \mathcal{N} \left(\langle c, \mu \rangle, c \Sigma c^T \right)$
- X_i and X_j are independent \iff $Cov(X_i, X_j) = 0$
- The standardized normal distribution is standard normal distributed

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

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

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

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

- Linear combinations are univariate normal distributed: $\langle c, X \rangle \sim \mathcal{N} \left(\langle c, \mu \rangle, c \Sigma c^T \right)$
- X_i and X_j are independent \iff $Cov(X_i, X_j) = 0$
- The standardized normal distribution is standard normal distributed

$$\Sigma^{-rac{1}{2}}\cdot(X-\mu)\sim\mathcal{N}_{d}(0,\mathbb{1})$$

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

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



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

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

• The sample mean \bar{Y} where $\bar{Y}_j := \frac{1}{n} \sum_{i=1}^n Y_{ij}$ is an estimator for μ .

Estimating μ and Σ

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

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

- 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

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

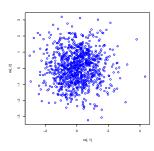
is an (unbiased) estimator for Σ .

Contents

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in R
- 6 Hotellings T²-test
- Testing for normality

- > library("mvtnorm") # multivariate t- and normal distrib.
- > Sigma0 <- matrix(c(1,0,0,1),ncol=2)</pre>
- > 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

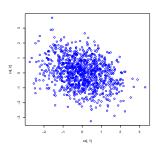
- > library("mvtnorm") # multivariate t- and normal distrib.
- > Sigma0 <- matrix(c(1,0,0,1),ncol=2)
- > dmvnorm(c(0,0),mean=c(0,0),sigma=Sigma0)
- [1] 0.1591549
- > dmvnorm(c(0,0)) # same, the standard normal is the default [1] 0.1591549
- > xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma0)
- > plot(xx[,1],xx[,2])



Correlation -0.3:

```
> Sigma3 <- matrix(c(1,-0.3,-0.3,1),ncol=2)
```

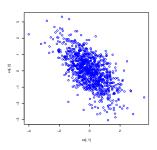
- > xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma3)
- > plot(xx[,1],xx[,2])



Correlation -0.6:

```
> Sigma6 <- matrix(c(1,-0.6,-0.6,1),ncol=2)
```

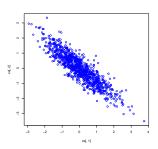
- > xx <- rmvnorm(1000,mean=c(0,0),sigma=Sigma6)
- > plot(xx[,1],xx[,2])



Correlation -0.9:

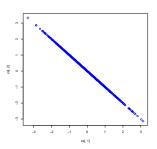
```
> Sigma9 <- matrix(c(1,-0.9,-0.9,1),ncol=2)
```

- > 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

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in F
- 6 Hotellings T²-test
- Testing for normality

• 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.

• 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.

• 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. 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.

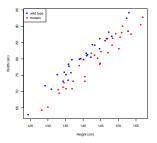
• Multivariate case: If $X := (X_{1,\cdot}, \dots, X_{n,\cdot})$ be a sample from the $\mathcal{N}_d(\mu, \Sigma)$ distribution with n > d, then the statistics

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

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 T²-test detect this difference?

- > raspberry <- read.table("raspberry.csv",sep=",",header=T)</pre>
- > raspberry.wt<-subset(raspberry,type=="wild type",select=-3)</pre>
- > raspberry.mu<-subset(raspberry,type=="mutant",select=-3)</pre>
- > 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 T²-test detects a significant difference

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

```
# alternative syntax with a formula
> 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
```

• The T²-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)

- The T²-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)
- The T²-test (like the t-test) is prone to dependence in the data. The test assumes that different samples (the rows of X) are independent. So be careful when sampling your data.

- The T²-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)
- The T²-test (like the t-test) is prone to dependence in the data. The test assumes that different samples (the rows of X) are independent. So be careful when sampling your data.
- The T^2 -test assumes n > d, so make sure that you have more samples than variables.

- The T²-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)
- The T²-test (like the t-test) is prone to dependence in the data. The test assumes that different samples (the rows of X) are independent. So be careful when sampling your data.
- The T^2 -test assumes n > d, so make sure that you have more samples than variables.
- The T²-test (more than the t-test) looses test power if the true distribution (of the population) deViates from the normal distribution (e.g., the Shapiro-Wilk test tests for normality)

- The T²-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)
- The T²-test (like the t-test) is prone to dependence in the data. The test assumes that different samples (the rows of X) are independent. So be careful when sampling your data.
- The T^2 -test assumes n > d, so make sure that you have more samples than variables.
- The T²-test (more than the t-test) looses test power if the true distribution (of the population) deViates from the normal distribution (e.g., the Shapiro-Wilk test tests for normality)
- If one of the variables shows already a significant difference between the group, then also the T²-test yields a significant difference.

Contents

- Motivating example
- Random vectors and random matrices
- Multivariate normal distribution
- The multivariate normal distribution in F
- 6 Hotellings T²-test
- Testing for normality

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

W = 0.9915, p-value = 0.7839