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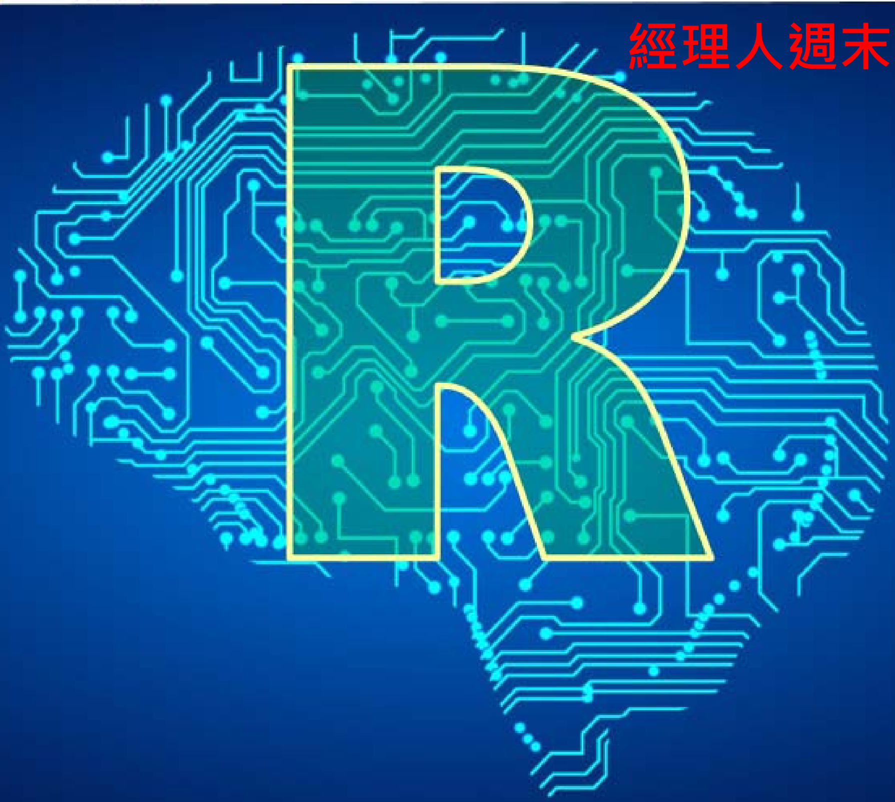

參數估計
與假設檢定

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本章大綱

- **參數估計 (parameter estimation)**
  (利用樣本統計量及其抽樣分配來對母體參數進行推估, 以瞭解母體的特性)
  - **點估計** (動差法、最大概似法、最小平方法)
    - 評斷準則: 不偏性、有效性、一致性、最小變異不偏性、充份性。
  - **區間估計**
  - **貝式估計法**

Frequentist parameter estimation

- 簡介統計假設檢定 (Hypothesis Testing)
- 平均數檢定 (t檢定): 單樣本、成對樣本、雙樣本
- 單因子變異數分析 (One-way Analysis of Variance, ANOVA)
- 無母數檢定 (Non-parametric Tests)
- Test for Normality
- Permutation Tests
- Chi-Square Test

The Likelihood Function

1. Suppose the sample are iid from a distribution with density function $\underline{\ f(X|\theta)\ }$, where θ is a parameter.

2. The **likelihood function** is the <u>conditional probability</u> of <u>observing the sample</u>, given $\underline{\ \theta\ }$

$$L(\theta) = \underline{\prod_{i=1}^{n} f(x_i|\theta)}\ .$$

   (a) The parameter could be a vector of parameters, $\theta = \underline{(\theta_1, \cdots, \theta_p)}$.

   (b) The likelihood function regards the <u>data</u> as a function of the <u>parameter θ</u>.

   (c) The **log likelihood** function

$$l(\theta) = \log(L(\theta)) = \underline{\sum_{i=1}^{n} \log f(x_i|\theta)}\ .$$

最大概似估計法
Maximum Likelihood Estimation

1. The method of maximum likelihood was introduced by **R.A. Fisher** (1890-1962, English statistician).

   (a) By maximizing the likelihood function $L(\theta)$ with respect to θ, we are looking for the most likely value of θ given the sample data.

   (b) Θ: parameter space of possible values of θ.

   (c) If the $\max L(\theta)$ exists and it occurs at a unique point $\hat{\theta} \in \Theta$, then $\hat{\theta}$ is called maximum likelihood estimator of θ.

$$\frac{\partial L(\theta)}{\partial \theta} = 0 \quad \text{且} \quad \frac{\partial^2 L(\theta)}{\partial \theta^2} < 0$$

點估計步驟：

1. 抽取代表性樣本
2. 選擇一個較佳的樣本統計量當估計式
3. 計算估計式的估計值
4. 以該估計值推論母體參數並作決策

MLE of (mu, sigma^2) from a normal population

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

$$X_1, \ldots, X_n \sim \text{i.i.d. } N(\mu, \sigma^2).$$

The probability density function for a sample of *n* independent identically distributed (iid) normal random variables (the likelihood) is

$$f(x_1, \ldots, x_n \mid \mu, \sigma^2) = \prod_{i=1}^{n} f(x_i \mid \mu, \sigma^2) = \left(\frac{1}{2\pi\sigma^2}\right)^{n/2} \exp\left(-\frac{\sum_{i=1}^{n}(x_i - \mu)^2}{2\sigma^2}\right),$$

$$\mathcal{L}(\mu, \sigma) = f(x_1, \ldots, x_n \mid \mu, \sigma)$$

$$\log(\mathcal{L}(\mu, \sigma)) = (-n/2)\log(2\pi\sigma^2) - \frac{1}{2\sigma^2}\sum_{i=1}^{n}(x_i - \mu)^2$$

$$0 = \frac{\partial}{\partial \mu}\log(\mathcal{L}(\mu, \sigma)) = 0 - \frac{-2n(\bar{x} - \mu)}{2\sigma^2}. \quad \Rightarrow \quad \hat{\mu} = \bar{x} = \sum_{i=1}^{n} \frac{x_i}{n}. \qquad E\left[\widehat{\mu}\right] = \mu$$

https://en.wikipedia.org/wiki/Maximum_likelihood_estimation

MLE of (mu, sigma^2) from a normal population

$$0 = \frac{\partial}{\partial \sigma} \log\left(\left(\frac{1}{2\pi\sigma^2}\right)^{n/2} \exp\left(-\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2 + n(\bar{x}-\mu)^2}{2\sigma^2}\right)\right)$$

$$= \frac{\partial}{\partial \sigma}\left(\frac{n}{2}\log\left(\frac{1}{2\pi\sigma^2}\right) - \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2 + n(\bar{x}-\mu)^2}{2\sigma^2}\right)$$

$$= -\frac{n}{\sigma} + \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2 + n(\bar{x}-\mu)^2}{\sigma^3}$$

$$\widehat{\sigma}^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \mu)^2. \qquad \mu = \widehat{\mu} \qquad \Rightarrow \qquad \widehat{\sigma}^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2$$

The maximum likelihood estimator

for $\theta = (\mu, \sigma^2)$ is $\hat{\theta} = \left(\widehat{\mu}, \widehat{\sigma}^2\right)$

$$E\left[\widehat{\sigma}^2\right] = \frac{n-1}{n}\sigma^2.$$

區間估計
(Interval Estimation)

- 區間估計是先對未知的母體參數求點估計值，然後在一信賴水準(Confidence Level) 下，導出一個上下區間，此區間稱為信賴區間(Confidence Interval)，信賴水準是指該區間包含母體參數的可靠度。
- 95% 信賴區間表示，做100 次信賴區間，區間約包含母體參數95 次

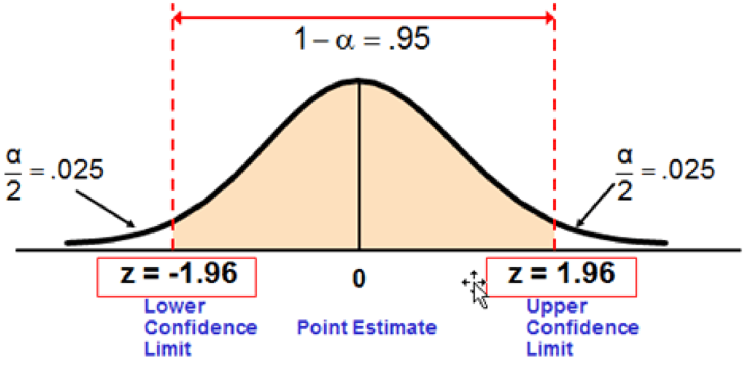
Interval Estimate of Population Mean

若大樣本(n> 30)、 母體σ已知,
由中央極限定理知 $\bar{X} \sim N(\mu, \sigma^2/n)$ ⇨ $Z = \dfrac{\bar{X}-\mu}{\sigma/\sqrt{n}}$

$$P(-z \le Z \le z) = 1-\alpha = 0.95.$$

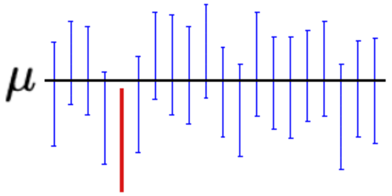
$$\Phi(z) = P(Z \le z) = 1 - \frac{\alpha}{2} = 0.975,$$

$$z = \Phi^{-1}(\Phi(z)) = \Phi^{-1}(0.975) = 1.96,$$



$$0.95 = 1-\alpha = P(-z \le Z \le z) = P\left(-1.96 \le \frac{\bar{X}-\mu}{\sigma/\sqrt{n}} \le 1.96\right)$$

$$= P\left(\bar{X} - 1.96\frac{\sigma}{\sqrt{n}} \le \mu \le \bar{X} + 1.96\frac{\sigma}{\sqrt{n}}\right).$$



A 95% confidence interval indicates that 19 out of 20 samples (95%) from the same population will produce confidence intervals that contain the population parameter.

範例: 老年人看電視的時間

根據行政院主計處調查，台灣地區15歲以上的人口中，以老年人(65歲以上)看電視的時間最長。現在新立傳播公司計畫推出老年人的電視節目，因此想要了解老年人看電視的時間，以決定電視節目的數量。新立公司於是採隨機抽樣法抽取台北市100位老人調查看電視的時數，結果得知，每星期看電視的平均時間為 21.2小時。假設根據過去數次調查的資料，已知每星期看電視時間的標準差為8小時，問在95%信賴水準下，每星期看電視平均時間的信賴區間為何？

信賴水準為95%，$\bar{X}$=21.2小時，σ =8小時， n =100

$\bar{X}$ 的抽樣分配為常態分配 $N \sim (\mu, \sigma_{\bar{X}}^2)$ ⇨ $P(|\bar{X} - \mu| \le 1.96\sigma_{\bar{X}}) = 0.95$

$$\sigma_{\bar{X}} = \frac{\sigma}{\sqrt{n}} = \frac{8}{\sqrt{100}} = 0.8$$

在 $1-\alpha$ 信賴水準下，母體平均數的信賴區間為

$$\bar{X} \pm Z_{\alpha/2}\sigma_{\bar{X}}$$

$\bar{X} \pm Z_{\alpha/2}\sigma_{\bar{X}} = 21.2 \pm 1.96 \times 0.8$ ⇨ $19.632 \le \mu \le 22.768$

可推論：「老年人每星期平均看電視的時間在 19.632~22.768小時之間，而此一區間的可信度(信賴水準)為95%。」

Bayesian Statistics
貝式統計

1. In the **frequentist approach** to statistics, the parameters of a distribution are considered to be fixed but unknown constants.

2. The **Bayesian approach** views the unknown parameters of a distribution as random variables.

   (a) In Bayesian analysis, probabilities can be computed for parameters as well as the sample statistics.

   (b) Bayes' Theorem allows one to revise the prior belief about an unknown parameter based on observed data.

Bayes' Theorem

1. If A and B are events and $P(B) > 0$, then

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}$$

2. The distributional form of Bayes' Theorem for continuous random variables is

$$f_{X|Y=y}(x) = \frac{f_{Y|X=x}(y)f_X(x)}{f_Y(y)} = \frac{f_{Y|X=x}(y)f_X(x)}{\int_{-\infty}^{\infty} f_{Y|X=x}(y)f_X(x)\ dx}$$

Bayesian Statistics
貝式統計

3. Suppose that X has the density $f(x|\theta)$.

(a) $f_\theta(\theta)$: the pdf of the prior distribution of θ.

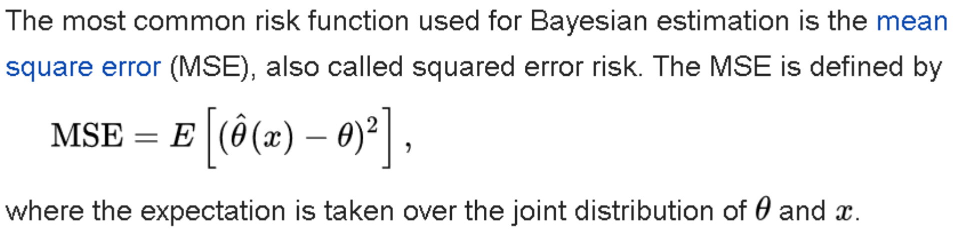
(b) The conditional density of θ given the sample observations $x_1, \cdots, x_n$ is called the posterior density

$$f_{\theta|x}(\theta) = \frac{f(x_1, \cdots, x_n|\theta) f_\theta(\theta)}{\int f(x_1, \cdots, x_n|\theta) f_\theta(\theta)\ d\theta}.$$

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}$$

(c) The posterior distribution summarizes our modified belief about the unknown parameters, taking into account the observed data.

(d) One is interested in computing posterior quantities such as posterior means, posterior modes, posterior standard deviations.



The most common risk function used for Bayesian estimation is the mean square error (MSE), also called squared error risk. The MSE is defined by

$$\mathrm{MSE} = E\left[(\hat{\theta}(x) - \theta)^2\right],$$

where the expectation is taken over the joint distribution of θ and x.

Bayes Estimator for the Mean of a Normal Distribution

$X_1, X_2, \ldots, X_n$ be a random sample $N(\mu, \sigma^2)$. μ is unknown and σ^2 is known.

prior distribution for μ is normal with mean μ_0 and variance σ_0^2

$$f(\mu) = \frac{1}{\sqrt{2\pi}\sigma_0} e^{-(\mu - \mu_0)^2/(2\sigma_0^2)} = \frac{1}{\sqrt{2\pi}\sigma_0^2} e^{-(\mu^2 - 2\mu_0 + \mu_0^2)/(2\sigma_0^2)}$$

The joint probability distribution of the sample

$$f(x_1, x_2, \ldots, x_n \,|\, \mu) = \frac{1}{(2\pi\sigma^2)^{n/2}} e^{-(1/2\sigma^2)\sum_{i=1}^{n}(x_i - \mu)^2}$$

$$= \frac{1}{(2\pi\sigma^2)^{n/2}} e^{-(1/2\sigma^2)\left(\sum x_i^2 - 2\mu \sum x_i + n\mu^2\right)}$$

the joint probability distribution of the sample and μ is

$$f(x_1, x_2, \ldots, x_n, \mu) = \frac{1}{(2\pi\sigma^2)^{n/2}\sqrt{2\pi\sigma_0}} e^{-(1/2)\left[\left(1/\sigma_0^2 + n/\sigma^2\right)\mu^2 - \left(2\mu_0/\sigma_0^2 + 2\sum x_i/\sigma^2\right)\mu + \sum x_i^2 \;/\sigma^2 + \mu_0^2\;/\sigma_0^2\right]}$$

$$= e^{-(1/2)\left[\left(\frac{1}{\sigma_0^2} + \frac{1}{\sigma^2/n}\right)\mu^2 - 2\left(\frac{\mu_0}{\sigma_0^2} + \frac{\bar{x}}{\sigma^2/n}\right)\mu\right]} h_1(x_1, \ldots, x_n, \sigma^2, \mu_0, \sigma_0^2)$$

Bayes Estimator for the Mean of a Normal Distribution

$$f(x_1, x_2,\ldots, x_n, \mu) = e^{-(1/2)\left[\left(\frac{1}{\sigma_0^2}+\frac{1}{\sigma^2/n}\right)\mu^2-2\left(\frac{\mu_0}{\sigma_0^2}+\frac{\bar{x}}{\sigma^2/n}\right)\mu\right]} h_1(x_1,\ldots,x_n,\sigma^2,\mu_0,\sigma_0^2)$$

$$f\left(x_1, x_2,\ldots, x_n, \mu\right) = e^{-(1/2)\left(\frac{1}{\sigma_0^2}+\frac{1}{\sigma^2/n}\right)\left[\mu^2-\left(\frac{(\sigma^2/n)\,\mu_0}{\sigma_0^2+\sigma^2/n}+\frac{\bar{x}\sigma_0^2}{\sigma_0^2+\sigma^2/n}\right)\right]^2} h_2(x_1,\ldots,x_n,\sigma^2,\mu_0,\sigma_0^2)$$

$h_i(x_1, \ldots, x_n, \sigma^2, \mu_0, \sigma_0^2)$ is a function of the observed values and the parameters σ^2, μ_0, and σ_0^2.

because $f(x_1, \ldots, x_n)$ does not depend on μ,

$$f\left(\mu|x_1,\ldots,x_n\right) = e^{-(1/2)\left(\frac{1}{\sigma_0^2}+\frac{1}{\sigma^2/n}\right)\left[\mu^2-\left(\frac{\left(\sigma^2/n\right)\mu_0+\sigma_0^2\bar{x}}{\sigma_0^2+\sigma^2/n}\right)\right]} h_3\left(x_1,\ldots,x_n,\sigma^2,\mu_0,\sigma_0^2\right)$$

a normal probability density function

posterior mean $\dfrac{\left(\sigma^2/n\right)\mu_0+\sigma_0^2\,\bar{x}}{\sigma_0^2+\sigma^2/n}$

posterior variance $\left(\dfrac{1}{\sigma_0^2}+\dfrac{1}{\sigma^2/n}\right)^{-1} = \dfrac{\sigma_0^2\left(\sigma^2/n\right)}{\sigma_0^2+\sigma^2/n}$

Bayes Estimator for the Mean of a Normal Distribution

posterior mean $\dfrac{(\sigma^2/n)\mu_0 + \sigma_0^2\,\overline{x}}{\sigma_0^2 + \sigma^2/n}$

suppose that we have a sample of size $n = 10$ from

from a normal distribution with unknown mean μ and variance $\sigma^2 = 4$.

Assume that the prior distribution for μ is normal with mean $\mu_0 = 0$ and variance $\sigma_0^2 = 1$.

If the sample mean is 0.75, the Bayes estimate of μ is

$$\frac{(4/10)0 + 1(0.75)}{1 + (4/10)} = \frac{0.75}{1.4} = 0.536$$

Hypothesis Testing (1)

Hypothesis Test

a procedure for determining if an assertion about a characteristic of a population is reasonable.

Example

"average price of a gallon of regular unleaded gas in Massachusetts is $2.5"

Is this statement true?

- find out every gas station.
- find out a small number of randomly chosen stations.

CITGO
2.23 9/10 REGULAR UNLEADED
2.33 9/10 MID GRADE UNLEADED
2.43 9/10 PREMIUM UNLEADED

Sample average price was $2.2.

- Is this 30 cent difference a result of chance variability, or
- is the original assertion incorrect?

Hypothesis Testing (2)

null hypothesis:

- H_0: μ = 2.5. (the average price of a gallon of gas is $2.5)
- H_0: $\mu_A - \mu_B = \mu_0$.

alternative hypothesis:

- H_a: $\mu > 2.5$. (gas prices were actually higher)
- H_a: $\mu < 2.5$.
- H_a: μ != 2.5.

significance level (alpha):

- Decide in advance.
- Alpha = 0.05: the probability of incorrectly rejecting the null hypothesis when it is actually true is 5%.

Hypothesis Testing (3)

Biological Question → ***Statistical Formulation***

H_0: No differential expressed.

H_0: no difference in the mean gene expression in the group tested.

H_0: The gene will have equal means across every group.

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$ $(\ldots = \mu_n)$

H_0: no differential expressed.

- The test is significant

  = Reject **H_0**

- False Positive

  = (Reject **H_0** | **H_0** true)

  = concluding that a gene is differentially expressed when in fact it is not.

- **A p-value=0.05 indicates that you would have only a 5% chance of drawing the sample being tested if the null hypothesis was actually true.**

- **The p-value is the smallest level of significance at which a null hypothesis may be rejected**

The *p*-values

p-values

- probability of **false positives** (Reject **H_0 | H_0** true).
- probability of observing your data under the assumption that the null hypothesis is true.
- *p-value* = 0.03: only a 3% chance of drawing the sample if the null hypothesis was true.

Decision Rule

- Reject H_0 if *p-value* is less than alpha.
- $P < 0.05$ commonly used. (Reject **H_0,** the test is significant)
- The lower the *p-value*, the more significant.

p-value 的定義是：在已知(現有)的抽樣樣本下，能棄卻 H_0(虛無假設)的最小顯著水準。

p-value：若(前提) H_0 為真，則 test statistic 出現的可能性。(若p-value越小，表示抽樣樣本越(極端)不可能出現，因此推翻前提，拒絕H_0)。

p-value：以現有的抽樣所進行的推論，可能犯 type I error 的機率。(若p-value越小，表示拒絕H_0不太可能錯，因此拒絕H_0)。

林澤民，看電影學統計: p值的陷阱
(The Pitfalls of p-Values)
http://blog.udn.com/nilnimest/84404190
社會科學論叢2016年10月第十卷第二期
社會科學前沿課題論壇

"只要是使用正確的意義，p-value並沒有問題，只是不要去誤用它。不要只是著重在統計顯著性，因為model對錯的機率跟p-value不一樣。要使用p-value作檢定，要把它跟α來做比較，所以問題不只是p-value，而是α。界定了α之後，才知道結果是不是顯著。當得到一個顯著的結果以後，必須再來衡量偽陽性反機率的問題，也就是model後設機率的問題，這就不是p-value可以告訴你的。"

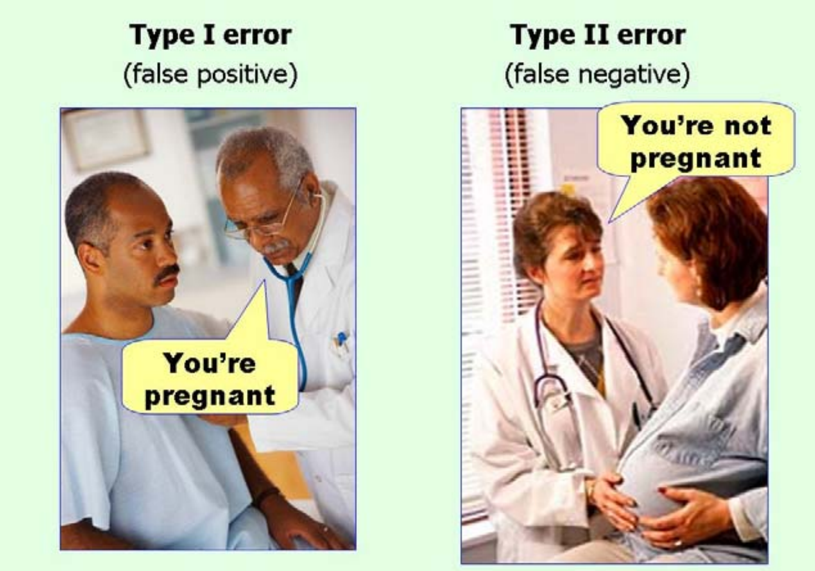
Type of Errors

Type I Error (alpha)

calling genes as differentially expressed when they are NOT (when you see things that are not there.)

Type II Error

NOT calling genes as differentially expressed when they ARE (when you dont see things that are there)



https://effectsizefaq.com/category/type-i-error/

Hypothesis Testing		Truth	
		H_0	H_1
Decision	Reject H_0	Type I Error (alpha) (false positive)	Right Decision (true positive)
	Don't Reject H_0	Right Decision	Type II Error (beta)

H_0: Not Pregnant

$$\text{Power} = 1 - \beta\,.$$

平均數檢定 in R

Hypothesis Testing	One Sample	Two Samples		> two Groups
	-	Paired data	Unpaired data	Complex data

Parametric (variance equal)	t-test
	t.test(x, mu = 0)
		t-test
		t.test(x-y, var.equal = TRUE)
		t.test(x, y, paired = TRUE, var.equal = TRUE)
			t-test
			t.test(x, y, var.equal = TRUE)
				One-Way Analysis of Variance (ANOVA)
				aov(x~g, data)
				oneway.test(x~g, data, var.equal = TRUE)

Parametric (variance not equal)		Welch t-test
		t.test(x-y)
		t.test(x, y, paired = TRUE)
			Welch t-test
			t.test(x, y)
				Welch ANOVA
				oneway.test(x~g, data)

Non-Parametric (無母數檢定)	Wilcoxon Signed-Rank Test
	wilcox.test(x, mu = 0)
		Wilcoxon Signed-Rank Test
		wilcox.test(x-y)
		wilcox.test(x, y, paired = TRUE)
			Wilcoxon Rank-Sum Test (Mann-Whitney U Test)
			wilcox.test(x, y)
				Kruskal-Wallis Test
				kruskal.test(x, g)

`pairwise.t.test {stats}`: Calculate pairwise comparisons between group levels with corrections for multiple testing

`TukeyHSD {stats}`: Compute Tukey Honest Significant Differences

Steps of Hypothesis Testing

1. Determine the null and alternative hypothesis, using mathematical expressions if applicable.
2. Select a significance level (alpha).
3. Take a random sample from the population of interest.
4. Calculate a test statistic from the sample that provides information about the null hypothesis.
5. Decision

Hypothesis Testing:

two-sided z-test & p-value

H_0: μ=35 null hypothesis

H_1: $\mu \neq$35 alternative hypothesis (μ>35; μ<35) one-sided

α signifcant level : =0.05

test statistic $z = \dfrac{\bar{X} - \mu}{\sigma/\sqrt{n}}$

Reject H0 if $|z| > z_{0.05}$

$H_0 : \mu = m$

$H_1 : \mu \neq m$

$\alpha = P_{H_0}(|Z| > z_{\alpha/2})$

Sample Data: =33.6

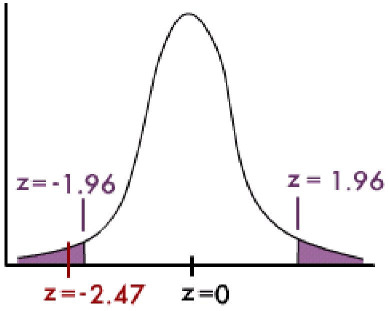
test statistic: z=-2.47

$(1-\alpha)100\%$ Confidence Interval:

$$P(z_{\alpha/2} < Z < z_{1-\alpha/2}) = 1 - \alpha$$

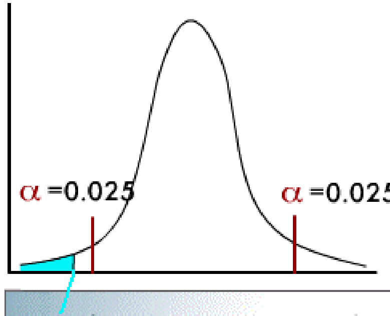
$$\text{p-value} = P_{H_0}(|Z| > z_0),\ z_0 = \frac{\bar{X}-m}{\sigma/\sqrt{n}}$$

The Classical Approach



Conclusion: since the z value of the test statistic (-2.47) is less than the critical value of z= -1.96, we reject the null hypothesis.

The *P*-Value Approach



P -value = 0.0068 times 2 (for a 2-sided test) = 0.0136

Conclusion: since the *P* -value of 0.0136 is less than the significance level of α=0.05, we reject the null hypothesis.

One Sample t-test

Assumption: the variable is normally distributed.

One sample t-test

$H_0 : \mu = \mu_0$

$H_1 : \mu \neq \mu_0$ (two-tailed).

μ: population mean.

α: significant level (e.g., 0.05).

Test Statistic:

$$T = \frac{\bar{X} - \mu}{S/\sqrt{n}}, \quad t_0 = \frac{\bar{X} - \mu_0}{S/\sqrt{n}}$$

$\bar{X}$: sample mean.

S: sample standard deviation.

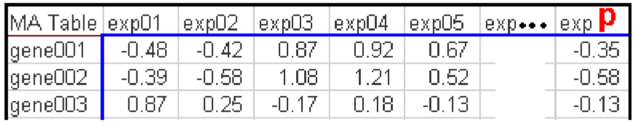
n: number of observations in the sample.

- Reject H_0 if $|t_0| > t_{\alpha/2, n-1}$.
- Power $= 1 - \beta$.
- $(1-\alpha)100\%$ Confidence Interval for μ:
  $\bar{X} - t_{\alpha/2} S/\sqrt{n} \leq \mu < \bar{X} + t_{\alpha/2} S/\sqrt{n}$
- p-value $= P_{H_0}(|\mathbf{T}| > t_0)$, $\mathbf{T} \sim t_{n-1}$.

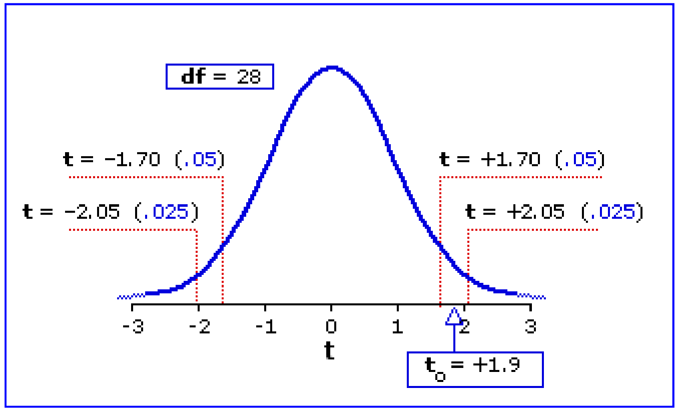
Question

■ whether a gene is differentially expressed for a condition with respect to baseline expression?

■ H_0: μ=0 (log ratio)



MA Table	exp01	exp02	exp03	exp04	exp05	exp•••	exp p
gene001	-0.48	-0.42	0.87	0.92	0.67		-0.35
gene002	-0.39	-0.58	1.08	1.21	0.52		-0.58
gene003	0.87	0.25	-0.17	0.18	-0.13		-0.13



Two Sample t-test

Paired Sample t-test

$H_0 : \mu_d = \mu_0$

$H_1 : \mu_d \neq \mu_0$ (two-tailed).

μ_d: mean of population differences.

α: significant level (e.g., 0.05).

Test Statistic:

$$T_d = \frac{\bar{d} - \mu_d}{S_d/\sqrt{n}}, \quad t_d = \frac{\bar{d} - \mu_0}{S_d/\sqrt{n}}$$

$\bar{d}$: average of sample differences.

S_d: standard deviation of sample difference

n: number of pairs.

- Reject H_0 if $|t_d| > t_{\alpha/2, n-1}$.
- Power $= 1 - \beta$.
- $(1-\alpha)100\%$ Confidence Interval for μ_d:
  $\bar{d} - t_{\alpha/2} S/\sqrt{n} \leq \mu_d < \bar{d} + t_{\alpha/2} S/\sqrt{n}$
- $p\text{-}value = P_{H_0}(|\mathbf{T}| > t_d)$, $\mathbf{T} \sim t_{n-1}$.

Two Sample t-test (Unpaired)

$H_0 : \mu_x - \mu_y = \mu_0$

$H_0 : \mu_x - \mu_y \neq \mu_0$

α: significant level (e.g., 0.05).

Test Statistic:

$$t_0 = \frac{(\bar{X} - \bar{Y}) - \mu_0}{\sqrt{\frac{S_x^2}{n} + \frac{S_y^2}{m}}}$$

for homogeneous variances:
$df = n + m - 2$

for heterogeneous variances:
adjusted df

Reject H_0 if $|t_0| > t_{\alpha/2,\, df}$

Assumptions of t-test

Be Normal

- paired t-test,
  the distribution of the subtracted data that must be normal.
- unpaired t-test,
  the distribution of both data sets must be normal.

How to Detect Normality

- **Plots**: Histogram, Density Plot, QQplot,…
- **Test for Normality**: Jarque-Bera test, Lilliefors test, Kolmogorov-Smirnov test.

Homogeneous

- the variances of the two population are equal.
- Test for equality of the two variances: Variance ratio F-test.

t.test {stats}: Student's t-Test

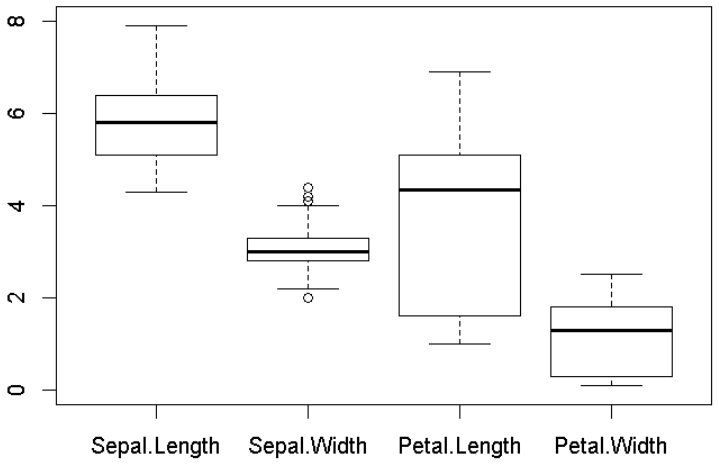
Description: Performs one and two sample t-tests on vectors of data.

Usage:
```
t.test(x, y = NULL,
         alternative = c("two.sided", "less", "greater"),
         mu = 0, paired = FALSE, var.equal = FALSE,
         conf.level = 0.95, ...)
```

```
> x <- iris$Sepal.Length
> y <- iris$Petal.Length
> alpha <- 0.05
> (vt <- (var.test(x, y)$p.value <= alpha))
[1] TRUE
> t.test(x, y, var.equal = !vt )

        Welch Two Sample t-test

data:  x and y
t = 13.098, df = 211.54, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 1.771500 2.399166
sample estimates:
mean of x mean of y
 5.843333  3.758000
```



`var.test {stats}`: Performs an F test to compare the variances of two samples from normal populations.
H_0: the ratio of the variances of the populations from which x and y were drawn, or in the data to which the linear models x and y were fitted, is equal to ratio=1.

Other t-Statistics

B-statistic

Lonnstedt and Speed, Statistica Sinica 2002: parametric empirical Bayes approach.

- B-statistic is an estimate of the posterior log-odds that each gene is DE.
- B-statistic is equivalent for the purpose of ranking genes to the penalized t-statistic $t = \frac{\bar{M}}{\sqrt{(a+s^2)/n}}$, where a is estimated from the mean and standard deviation of the sample variances s^2.

$$M_{gj}|\mu_g, \sigma_g \sim N(\mu_g, \sigma_g^2)$$

$$B_g = \log \frac{P(\mu_g \neq 0|M_{gj})}{P(\mu_g = 0|M_{gj})}$$

Lonnstedt, I. and Speed, T.P. Replicated microarray data. *Statistica Sinica* , 12: 31-46, 2002

Penalized t-statistic

Tusher et al (2001, PNAS, SAM)

Efron et al (2001, JASA)

$$t = \frac{\bar{M}}{(a+s)/\sqrt{n}}$$

General Penalized t-statistic

(Lonnstedt et al 2001)

$$t = \frac{b}{s^* \times SE}$$

multiple regression model

Penalized two-sample t-statistic

$$t = \frac{\bar{M}_A - \bar{M}_B}{s^* \times \sqrt{1/n_A + 1/n_B}}, \quad \text{where } s^* = \sqrt{a + s^2}$$

Robust General Penalized t-statistic

單因子變異數分析 (One-Way ANOVA)

- ANOVA can be considered to be a generalization of the *t*-test, when
  - compare more than two groups (e.g., *drug 1*, *drug 2*, and *placebo*), or
  - compare groups created by more than one independent variable while controlling for the separate influence of each of them (e.g., *Gender*, *type of Drug*, and *size of Dose*).

- One-way ANOVA compares groups using one parameter.
- ANOVA can test the following:
  - Are all the means from more than two populations equal?
  - Are all the means from more than two treatments on one population equal?
  - (This is equivalent to asking whether the treatments have any overall effect.)

One-Way ANOVA

- Assumptions
  - The subjects are sampled randomly.
  - The groups are independent.
  - The population variances are homogenous.
  - The population distribution is normal in shape.

- As with t-tests, violation of homogeneity is particularly a problem when we have quite different sample sizes.

- Homogeneity of variance test
  - Bartlett's test (1937)
  - Levene's test (Levene 1960)
  - O'Brien (1979)
  - …

ANOVA Table

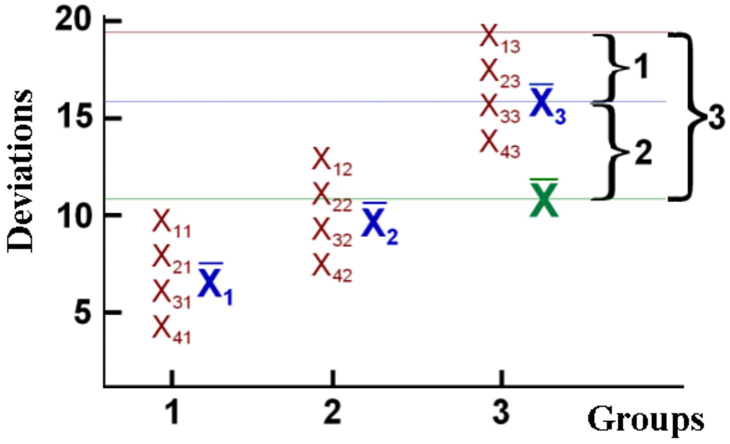
Groups

1	2	. . . j . . .	k
X_{11}	X_{12}	$\cdots$ X_{1j} $\cdots$	X_{1k}
X_{21}	X_{22}	$\cdots$ X_{2j} $\cdots$	X_{2k}
		$\cdots$	
X_{i1}	X_{i2}	$\cdots$ X_{ij} $\cdots$	X_{ik}
$\vdots$			$X_{n_k k}$
	$X_{n_2 2}$	$\cdots$ $\vdots$ $\cdots$	
$X_{n_1 1}$			
		$X_{n_j j}$	

$$T_j = \sum_{i=1}^{n_j} X_{ij} \qquad \bar{X}_j = \frac{T_j}{n_j}$$

$$T = \sum_{j=1}^{k} T_j \qquad \bar{X} = \frac{T}{N}$$

$$S^2 = \sum_{j=1}^{k} \sum_{i=1}^{n_j} \frac{(X_{ij} - \bar{X})^2}{N-1}$$



$$(X_{ij} - \bar{X}) = (X_{ij} - \bar{X}_j) + (\bar{X}_j - \bar{X})$$

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_k$$

$$X_{ij} = \mu_j + \epsilon_{ij} \qquad i = 1, \cdots, n_j$$
$$j = 1, \cdots, k$$

$$\epsilon_{ij} \sim N(0, \sigma^2)$$

$$\sum_{j=1}^{k} \sum_{i=1}^{n_j} (X_{ij} - \bar{X})^2 = \sum_{j=1}^{k} \sum_{i=1}^{n_j} [(X_{ij} - \bar{X}_j) + (\bar{X}_j - \bar{X})]^2$$

$$\sum_{j=1}^{k} \sum_{i=1}^{n_j} (X_{ij} - \bar{X})^2 = \sum_{j=1}^{k} \sum_{i=1}^{n_j} (X_{ij} - \bar{X}_j)^2 + \sum_{j=1}^{k} \sum_{i=1}^{n_j} (\bar{X}_j - \bar{X})^2$$

ANOVA Table

Source	SS	df	MS	F	p
Between	SS_B	$p-1$	MS_B	MS_B/MS_W	< 0.05
Within	SS_W	$N-p$	MS_W		
Total	SS_T	$N-1$			

$$SS_{Total} = SS_{Within} + SS_{Between}$$

$$F = \frac{MS_{Between}}{MS_{Within}}$$

Reject H_0, if $F_{obs} > F_{\{\alpha,k-1,N-k\}}$

Apply ANOVA to SRBCT data

- **khan {made4}**: Microarray gene expression dataset from Khan et al., 2001. Subset of 306 genes.
- http://svitsrv25.epfl.ch/R-doc/library/made4/html/khan.html
- Khan contains gene expression profiles of four types of small round blue cell tumours of childhood (SRBCT) published by Khan et al. (2001). It also contains further gene annotation retrieved from SOURCE at http://source.stanford.edu/.

```
> source("https://bioconductor.org/biocLite.R")
> biocLite("made4")
> library(made4)
> data(khan)
> # some EDA works should be done  before ANOVA
>
> # get the p-value from a anova table
> Anova.pvalues <- function(x){
+   x <- unlist(x)
+   SRBCT.aov.obj <- aov(x ~ khan$train.classes)
+   SRBCT.aov.info <- unlist(summary(SRBCT.aov.obj))
+   SRBCT.aov.info["Pr(>F)1"]
+ }
> # perform anova for each gene
> SRBCT.aov.p <- apply(khan$train, 1, Anova.pvalues)
```

Apply ANOVA to SRBCT data

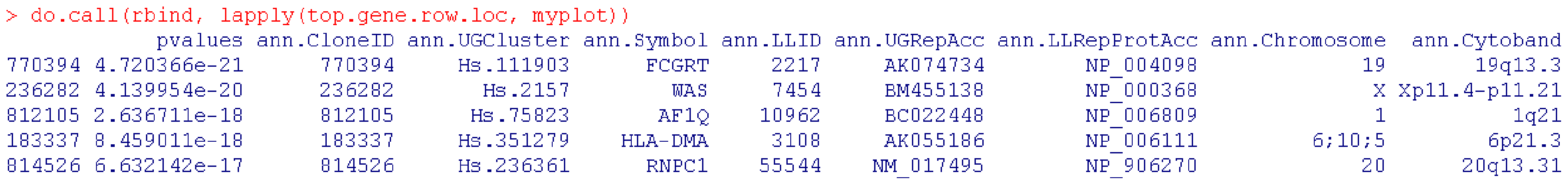
```
> # select the top 5 DE genes
> order.p <- order(SRBCT.aov.p)
> ranked.genes <- data.frame(pvalues=SRBCT.aov.p[order.p],
+                            ann=khan$annotation[order.p, ])
> top5.gene.row.loc <- rownames(ranked.genes[1:5,  ])
> # summarize the top5 genes
> summary(t(khan$train[top5.gene.row.loc, ]))
     770394           236282           812105           183337           814526      
 Min.   :0.0669   Min.   :0.0364   Min.   :0.1011   Min.   :0.0223   Min.   :0.1804  
 1st Qu.:0.3370   1st Qu.:0.1557   1st Qu.:0.3250   1st Qu.:0.1273   1st Qu.:0.4294  
 Median :0.6057   Median :0.2412   Median :0.7183   Median :0.2701   Median :0.6677  
 Mean   :1.5508   Mean   :0.3398   Mean   :1.1619   Mean   :0.5013   Mean   :0.9640  
 3rd Qu.:2.8176   3rd Qu.:0.3563   3rd Qu.:1.5543   3rd Qu.:0.5104   3rd Qu.:1.3620  
 Max.   :5.2958   Max.   :1.3896   Max.   :5.9451   Max.   :3.7478   Max.   :3.5809  
```

```
> # draw the side-by-side boxplot for top5 DE genes
> par(mfrow=c(1, 5), mai=c(0.3, 0.4, 0.3, 0.3))
> # get the location of xleft, xright, ybottom, ytop.
> usr <- par("usr")
> myplot <- function(gene){
+   # use unlist to convert "data.frame[1xp]" to "numeric"
+   boxplot(unlist(khan$train[gene, ]) ~ khan$train.classes,
+           ylim=c(0, 6), main=ranked.genes[gene, 4])
+   text(2, usr[4]-1, labels=paste("p=", ranked.genes[gene, 1],
+        sep=""), col="blue")
+   ranked.genes[gene,]
+ }
```

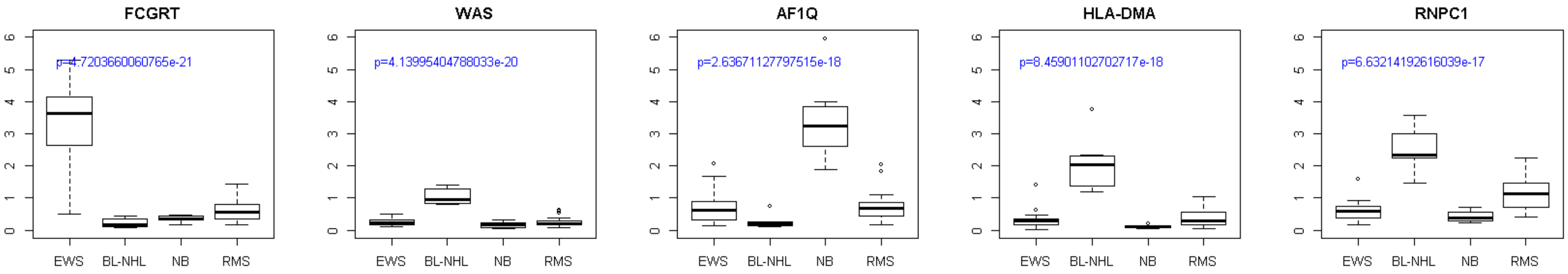
(重要技巧) 利用Key (gene.row.loc)
去連結多組資料(train, annotation)。

Apply ANOVA to SRBCT data

```
> # print the top5 DE genes info
> do.call(rbind, lapply(top5.gene.row.loc, myplot))
> # lappay returns "list" and use rbind to convert it to "data.frame"
> # Try sapply?
```



```
> do.call(rbind, lapply(top.gene.row.loc, myplot))
             pvalues ann.CloneID ann.UGCluster ann.Symbol ann.LLID ann.UGRepAcc ann.LLRepProtAcc ann.Chromosome  ann.Cytoband
770394 4.720366e-21      770394     Hs.111903      FCGRT     2217     AK074734        NP_004098             19      19q13.3
236282 4.139954e-20      236282       Hs.2157        WAS     7454     BM455138        NP_000368              X Xp11.4-p11.21
812105 2.636711e-18      812105      Hs.75823       AF1Q    10962     BC022448        NP_006809              1          1q21
183337 8.459011e-18      183337     Hs.351279    HLA-DMA     3108     AK055186        NP_006111         6;10;5        6p21.3
814526 6.632142e-17      814526     Hs.236361      RNPC1    55544    NM_017495        NP_906270             20      20q13.31
```

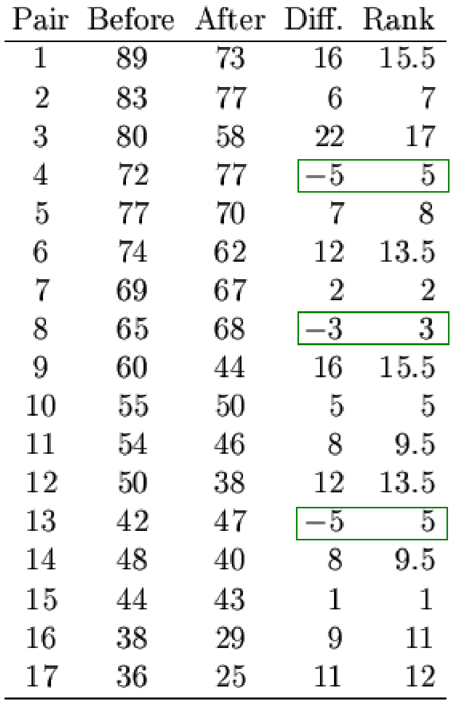


Non-parametric Statistics

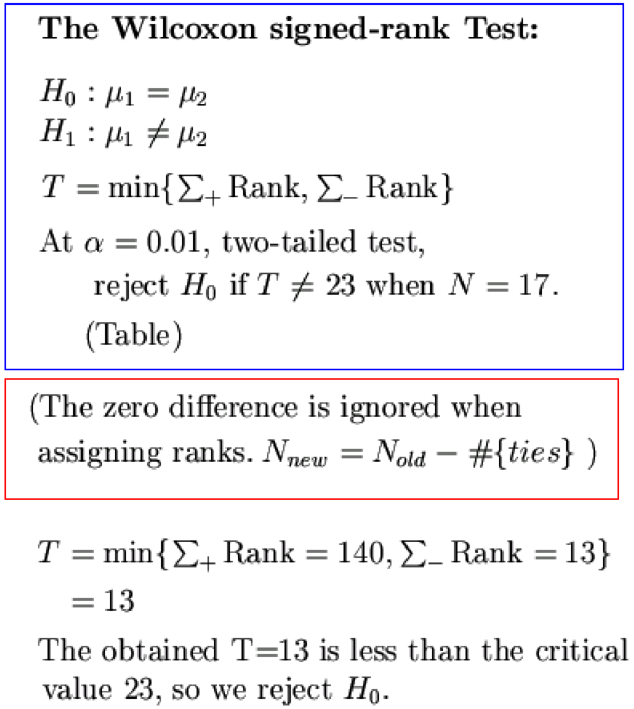
- Do not assume that the data is normally distributed.
- Nonparametric statistics is based on either being distribution-free or having a specified distribution but with the distribution's parameters unspecified.
- Nonparametric statistics includes both descriptive statistics and statistical inference.
- Non-parametric models: kernel density estimation, non-parametric regression, ...
- Non-parametric inferential statistical methods: Kolmogorov–Smirnov test, Kruskal–Wallis one-way analysis of variance, Mann–Whitney U test, Sign test, Wilcoxon signed-rank test,...

Wilcoxon Signed-Rank Test (paired)

- Null hypothesis: the population median from which both samples were drawn is the same.
  - The sum of the ranks for the "positive" (up-regulated) values is calculated and compared against a precomputed table to a p-value.
    - Sorting the absolute values of the differences from smallest to largest.
    - Assigning ranks to the absolute values.
    - Find the sum of the ranks of the positive differences.
  - If the null hypothesis is true, the sum of the ranks of the positive differences should be about the same as the sum of the ranks of the negative differences.



Pair	Before	After	Diff.	Rank
1	89	73	16	15.5
2	83	77	6	7
3	80	58	22	17
4	72	77	−5	5
5	77	70	7	8
6	74	62	12	13.5
7	69	67	2	2
8	65	68	−3	3
9	60	44	16	15.5
10	55	50	5	5
11	54	46	8	9.5
12	50	38	12	13.5
13	42	47	−5	5
14	48	40	8	9.5
15	44	43	1	1
16	38	29	9	11
17	36	25	11	12



The Wilcoxon signed-rank Test:

$H_0 : \mu_1 = \mu_2$
$H_1 : \mu_1 \neq \mu_2$

$T = \min\{\sum_+ \text{Rank}, \sum_- \text{Rank}\}$

At $\alpha = 0.01$, two-tailed test,
reject H_0 if $T \neq 23$ when $N = 17$.
(Table)

(The zero difference is ignored when assigning ranks. $N_{new} = N_{old} - \#\{ties\}$)

$T = \min\{\sum_+ \text{Rank} = 140, \sum_- \text{Rank} = 13\}$
$= 13$

The obtained T=13 is less than the critical value 23, so we reject H_0.

Parametric vs. Non-Parametric Test

Parametric Tests

- Assume that the data follows a certain distribution (normal distribution).
- Assuming equal variances and Unequal variances.
- More powerful.
- Not appropriate for data with outliers.

t-test	Non-parametric
Easy	Easy
Powerful	Robust
Widely Implemented	widely implemented
Not appropriate for data with outliers	Less powerful

Non-Parametric Tests

- When certain assumptions about the underlying population are questionable (e.g. normality).
- Does not assume normal distribution
- No variance assumption
- Ranks the order of raw/normalized data across conditions for analyses
- Decrease effects of outliers (Robust)
- Not recommended if there is less than 5 replicates per group
- Needs a high number of replicates
- Less powerful

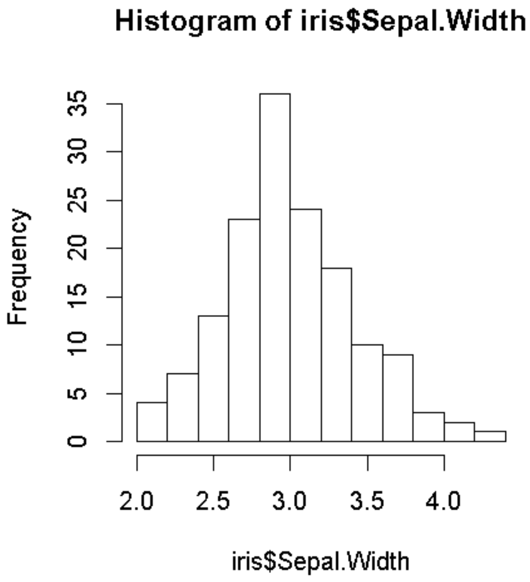
Formal Tests for Normality

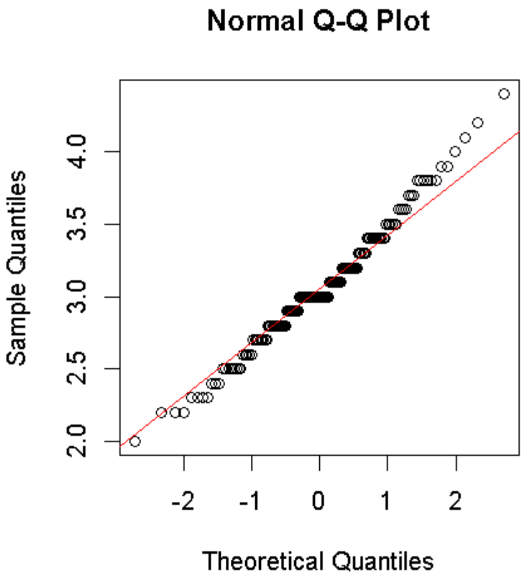
- The hypotheses used are:

H_0: The sample data are not significantly different than a normal population.

H_a: The sample data are significantly different than a normal population

```
> par(mfrow=c(1, 2))
> hist(iris$Sepal.Width)
> qqnorm(iris$Sepal.Width)
> qqline(iris$Sepal.Width, col="red")
```


Histogram of iris$Sepal.Width


Normal Q-Q Plot

Packages: `nortest`

Five omnibus tests for testing the composite hypothesis of normality:

`ad.test, cvm.test, lillie.test, pearson.test, sf.test`

ks.test, ad.test, shapiro.test

- Kolmogorov-Smirnov (K-S) test (Chakravarti et al., 1967).
- The Anderson-Darling test (Stephens, 1974).
- The Shapiro-Wilk normality test (Shapiro and Wilk, 1965).
- A large *p*-value (larger than, say, 0.05) indicates that the sample is not different from normal with the sample's mean and standard deviation.

```
> x <- iris$Sepal.Width
> ks.test(x, 'pnorm', mean(x), sd(x))

        One-sample Kolmogorov-Smirnov test

data:  x
D = 0.10566, p-value = 0.07023
alternative hypothesis: two-sided

Warning message:
In ks.test(x, "pnorm", mean(x), sd(x)) :
  ties should not be present for the Kolmogorov-Smirnov test
```

```
> library(nortest)
> ad.test(iris$Sepal.Width)

        Anderson-Darling normality test

data:  iris$Sepal.Width
A = 0.90796, p-value = 0.02023
```

```
> shapiro.test(iris$Sepal.Width)

        Shapiro-Wilk normality test

data:  iris$Sepal.Width
W = 0.98492, p-value = 0.1012
```

Which Normality Test Should I Use?

- Asghar Ghasemi and Saleh Zahediasl, Normality Tests for Statistical Analysis: A Guide for Non-Statisticians, *Int J Endocrinol Metab*. 2012 Spring; 10(2): 486–489.
  - assessing the normality assumption should be taken into account for using parametric statistical tests.
  - The K-S test, should no longer be used owing to its low power.
  - It is preferable that normality be assessed both visually and through normality tests, of which the **Shapiro-Wilk test** is highly recommended.

- NOTE:
  - If the data are not normal, use non-parametric tests.
  - If the data are normal, use parametric tests.
  - If you have groups of data, you MUST test each group for normality.
  - It's common seen that a model is built from the training data and is then applied to the testing data. Did these two data sets follow the same distribution?

Permutation Test

Coexpression of genes

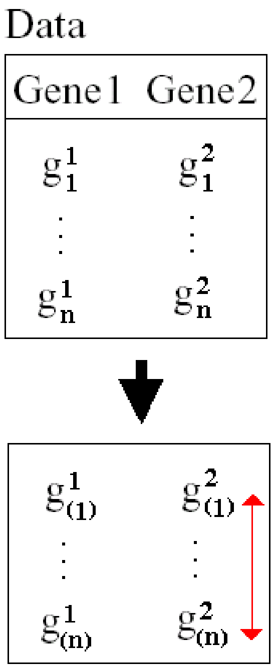
H_0: Gene 1 and Gene 2 are not correlated.

Test statistic T:

Pearson (or Spearman) correlation coefficient, calculate t_{obs}

Randomization: Under H_0 it is possible to permute the values observed for Gene 2. There are n! possibilities.

p-value: $p = P(T \ge t_{obs} \mid H_0) \approx \dfrac{\#\{\, T^* \ge t_{obs} \,\}}{n!}$



Data

$$\begin{array}{cc} \text{Gene1} & \text{Gene2} \\ \hline g_1^1 & g_1^2 \\ \vdots & \vdots \\ g_n^1 & g_n^2 \end{array}$$

$$\begin{array}{cc} g_{(1)}^1 & g_{(1)}^2 \\ \vdots & \vdots \\ g_{(n)}^1 & g_{(n)}^2 \end{array}$$

Random Permutation for group labels

Gene 1	Gene 2	Group		Group
1.4482	1.0709	1		2
0.4850	0.9324	1		1
1.1331	1.2379	1		4
		⋮		⋮
0.8015	0.6765	2		1
		⋮	➡	⋮
1.3726	1.2373	3		4
		⋮		⋮
1.1030	1.735	4		2
0.5148	1.0015	4		3

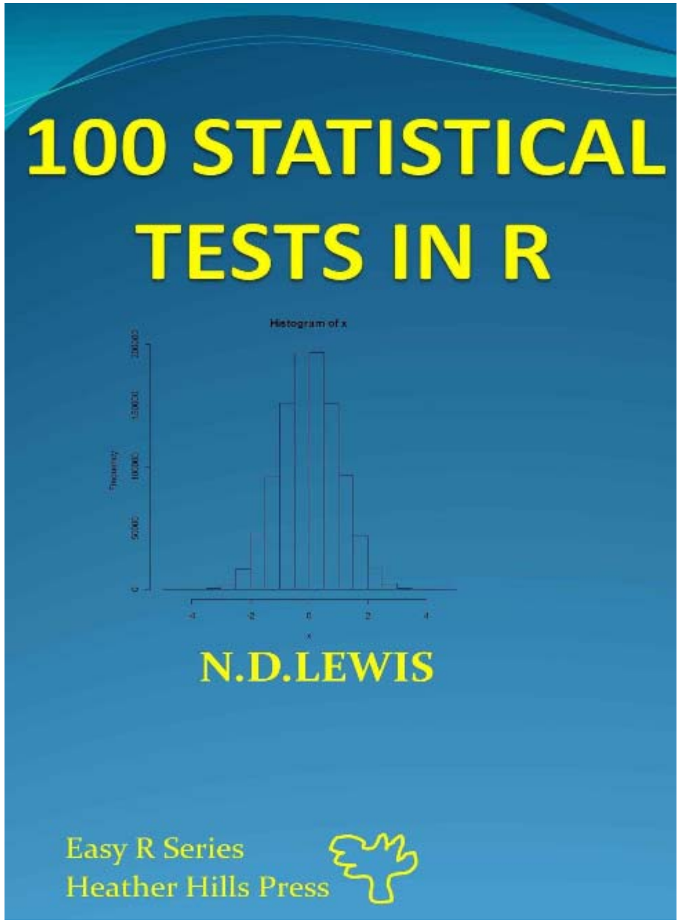
The permutation test allows determining the statistical significance of the score for every gene.

See also: the `coin` package and the `lmPerm` package:

`coin`: Conditional Inference Procedures in a Permutation Test Framework

`lmPerm`: Permutation Tests for Linear Models

卡方檢定: chisq.test



N.D Lewis, 100 Statistical Tests in R, Publisher: CreateSpace Independent Publishing Platform (April 15, 2013)

卡方檢定: chisq.test

- 適合度檢定(test of goodness of fit): 檢定資料是否符合某個比例關係或某個機率分佈
- 齊一性檢定(test of homogeneity): 檢定幾個不同類別中的比例關係是否一致
- 獨立性檢定(test of independence): 檢定兩個分類變數之間是否互相獨立。

`chisq.test {stats}`: Pearson's Chi-squared Test for Count Data

Description:

chisq.test performs chi-squared contingency table tests and goodness-of-fit tests.

Usage:

```
chisq.test(x, y = NULL, correct = TRUE, p =
rep(1/length(x), length(x)), rescale.p = FALSE,
simulate.p.value = FALSE, B = 2000)
```

Chi-Square Test for Independence

- H_0: In the population, the two categorical variables are independent.
- H_a: In the population, two categorical variables are dependent.

For testing independence in $I \times J$ contingency tables

$$H_0\colon \pi_{ij} = \pi_{i+}\pi_{+j} \quad \text{for all } i \text{ and } j$$

$\mu_{ij} = n\pi_{ij} = n\pi_{i+}\pi_{+j}$ as the expected frequency.

estimated expected frequencies.

$$\hat{\mu}_{ij} = np_{i+}p_{+j} = n\left(\frac{n_{i+}}{n}\right)\left(\frac{n_{+j}}{n}\right) = \frac{n_{i+}n_{+j}}{n}$$

The *Pearson chi-squared statistic* for testing H_0 is

$$X^2 = \sum \frac{(n_{ij} - \mu_{ij})^2}{\mu_{ij}}$$

The X^2 statistic has approximately a chi-squared distribution, for large n. **(WHY?)**

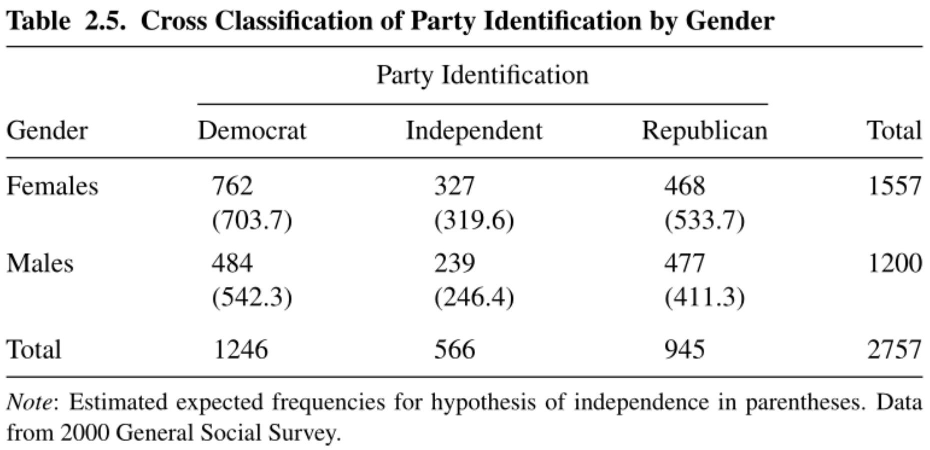


Table 2.5. Cross Classification of Party Identification by Gender

	Party Identification			
Gender	Democrat	Independent	Republican	Total
Females	762 (703.7)	327 (319.6)	468 (533.7)	1557
Males	484 (542.3)	239 (246.4)	477 (411.3)	1200
Total	1246	566	945	2757

Note: Estimated expected frequencies for hypothesis of independence in parentheses. Data from 2000 General Social Survey.

```
> M <- as.table(rbind(c(762, 327, 468),
                      c(484, 239, 477)))
> dimnames(M) <- list(gender = c("F", "M"),
+                     party = c("Democrat",
                                "Independent",
                                "Republican"))
> M
      party
gender Democrat Independent Republican
     F      762         327        468
     M      484         239        477
> (res <- chisq.test(M))
        Pearson's Chi-squared test

data:  M
X-squared = 30.07, df = 2, p-value = 2.954e-07
```