CENTRE DE RECHERCHE PUBLIC

Microarray Center

the Luxembourg Bioinformatics Network

UNIVERSITÉ DU LUXEMBOURG

STATISTICAL DATA ANALYSIS IN EXCEL

Lecture 5

Linear Regression

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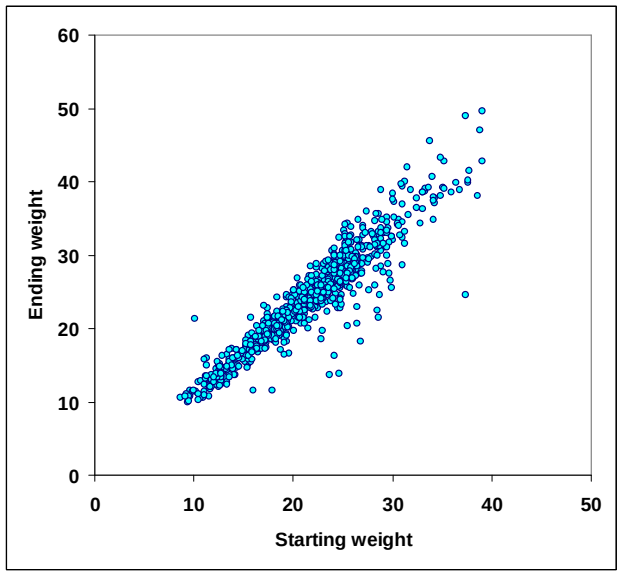
- **Introduction**
  - covariation and correlation measures
  - dependent and independent random variables
  - scatter plot and linear trendline
  - linear model
- **Testing for significance**
  - estimation of the noise variance
  - interval estimations
  - testing hypothesis about significance
- **Regression Analysis**
  - confidence and prediction
  - multiple linear regression
  - nonlinear regression

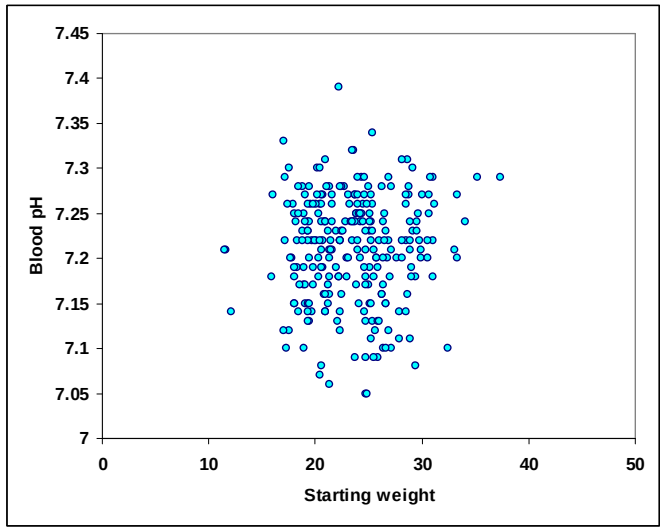
Dependent and Independent Variables

mice.xls

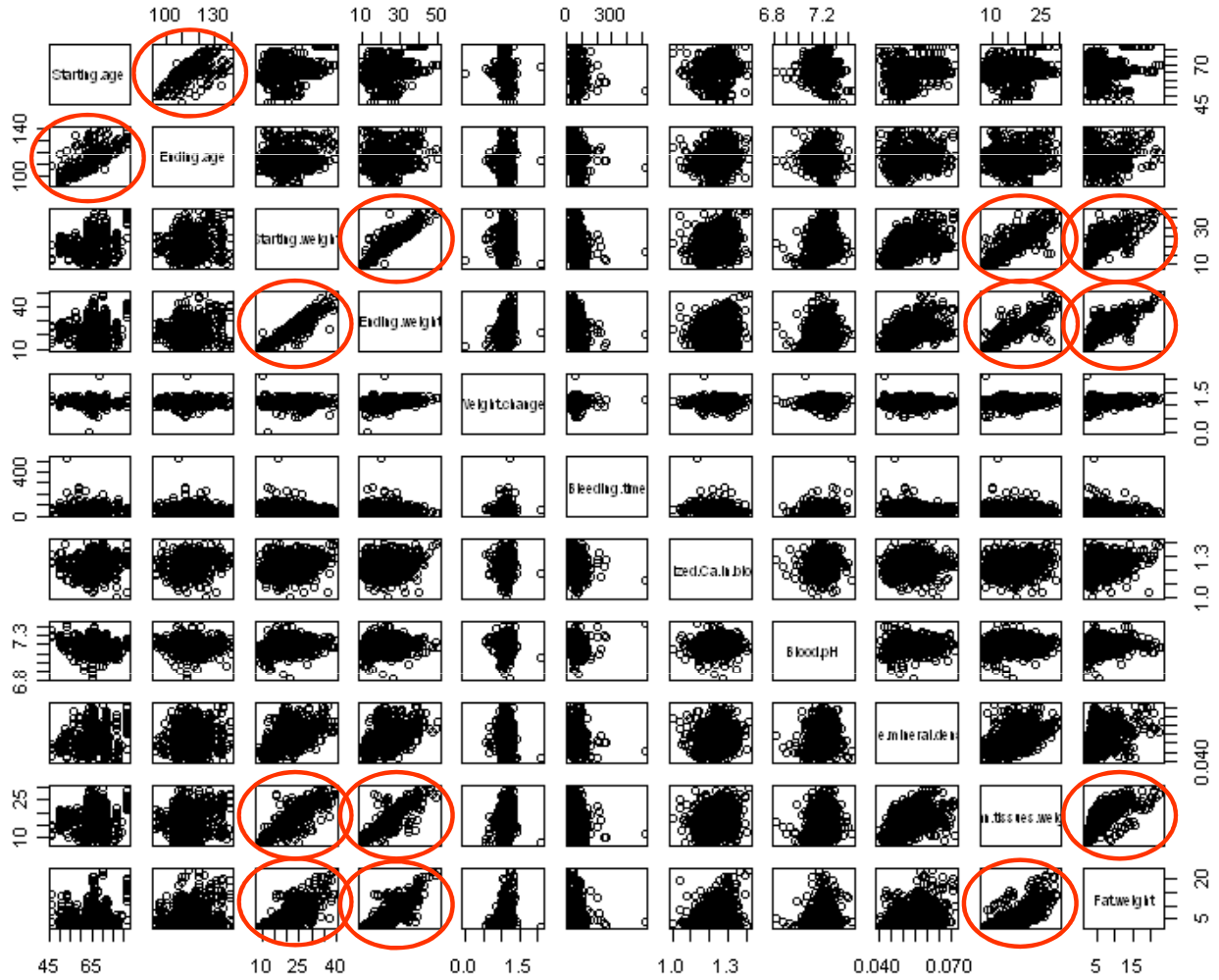
Ending weight vs. Starting weight



Blood pH vs. Starting weight



Dependent and Independent Variables



Measure of Association between 2 Variables

Covariance
A measure of linear association between two variables. Positive values indicate a positive relationship; negative values indicate a negative relationship.

population

$$\sigma_{xy} = \frac{\sum (x_i - \mu_x)(y_i - \mu_y)}{N}$$

sample

$$s_{xy} = \frac{\sum (x_i - m_x)(y_i - m_y)}{n-1}$$

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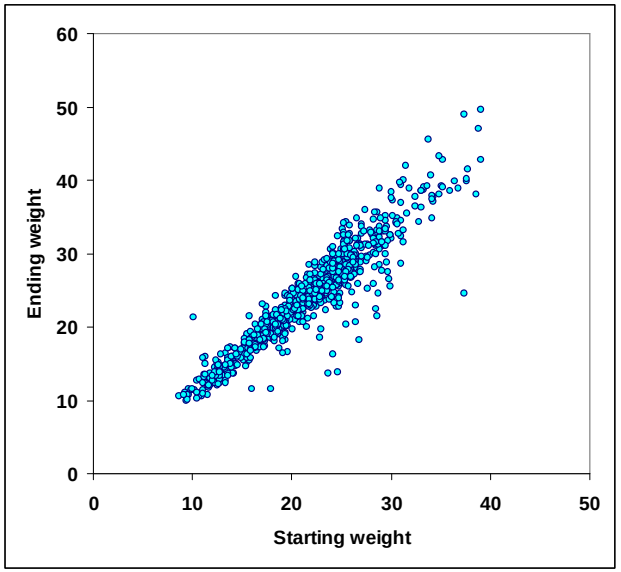


Ending weight vs. Starting weight

In Excel use function:

- `=COVAR(data)`

s_{xy} = 39.8

hard to interpret

Measure of Association between 2 Variables

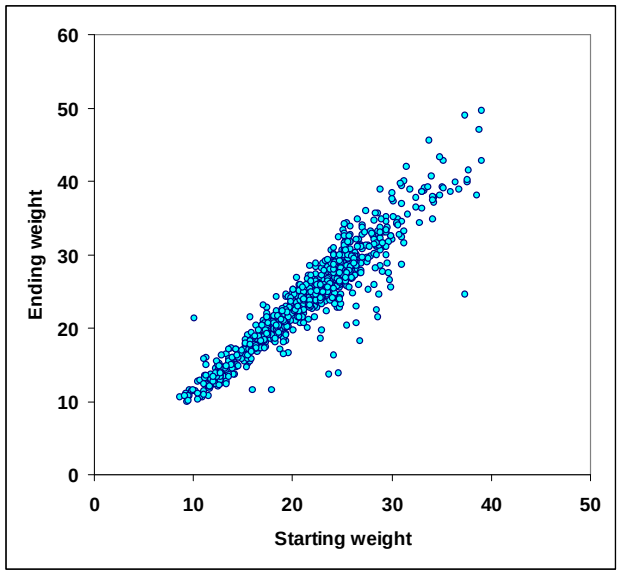
Correlation (Pearson product moment correlation coefficient)
A measure of linear association between two variables that takes on values between -1 and +1. Values near +1 indicate a strong positive linear relationship, values near -1 indicate a strong negative linear relationship; and values near zero indicate the lack of a linear relationship.

population

$$\rho_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y} = \frac{\sum (x_i - m_x)(y_i - m_y)}{\sigma_x \sigma_y N}$$

sample

$$r_{xy} = \frac{s_{xy}}{s_x s_y} = \frac{\sum (x_i - m_x)(y_i - m_y)}{s_x s_y (n-1)}$$

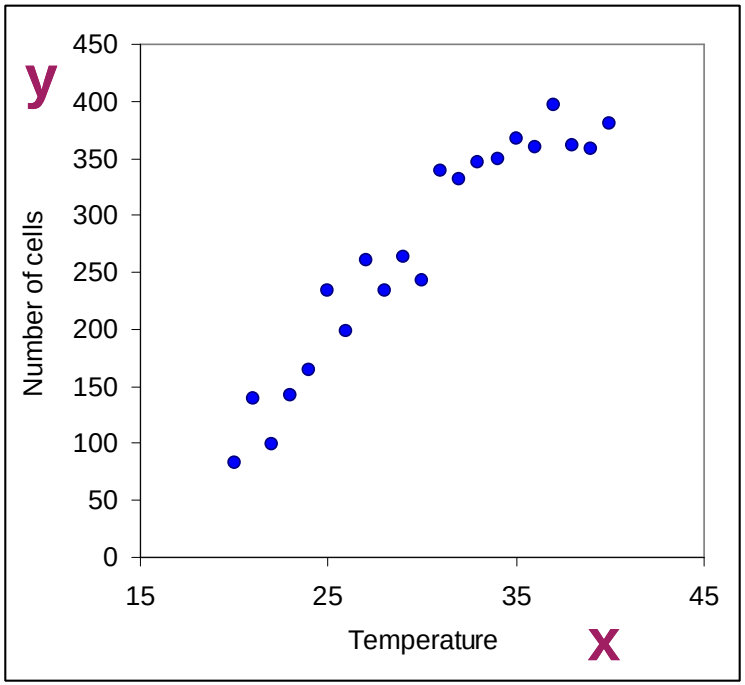


In Excel use function:

- `=CORREL(data)`

r_{xy} = 0.94

Temperature	Cell Number
20	83
21	139
22	99
23	143
24	164
25	233
26	198
27	261
28	235
29	264
30	243
31	339
32	331
33	346
34	350
35	368
36	360
37	397
38	361
39	358
40	381



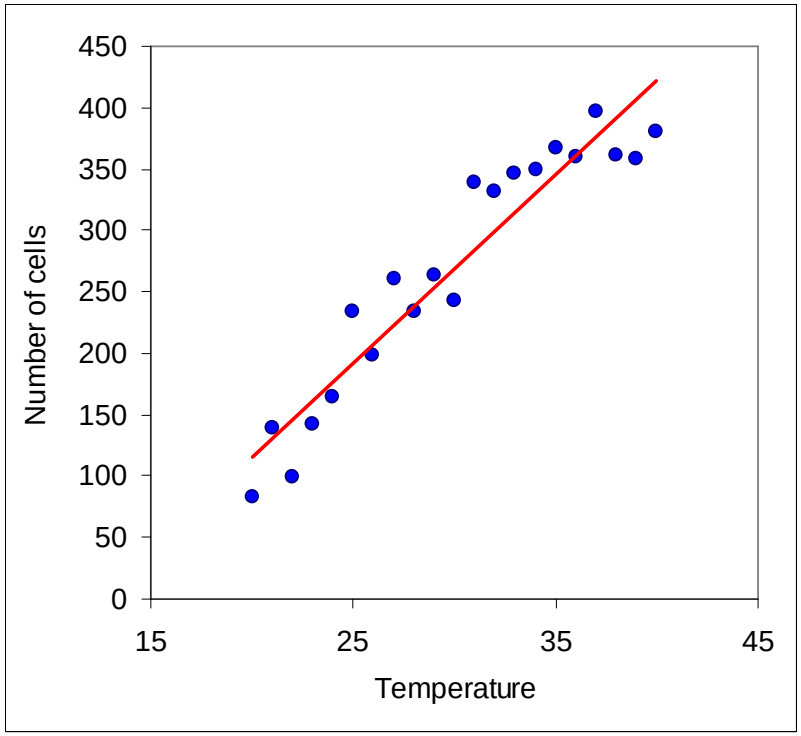
Cells are grown under different temperature conditions from 20° to 40°. A researched would like to find a dependency between T and cell number.

Dependent variable
The variable that is being predicted or explained. It is denoted by ***y***.

Independent variable
The variable that is doing the predicting or explaining. It is denoted by ***x***.

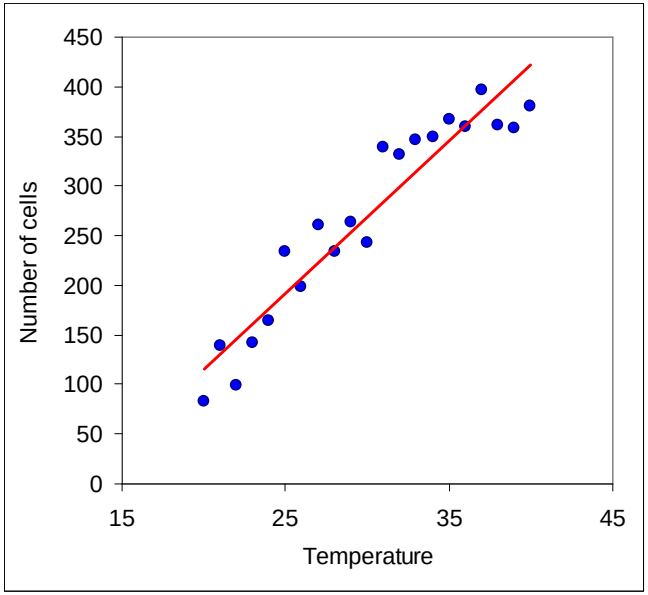
Simple linear regression
Regression analysis involving one independent variable and one dependent variable in which the relationship between the variables is approximated by a straight line.

- Building a *regression* means finding and tuning the model to explain the behaviour of the data



Regression model
The equation describing how y is related to x and an error term; in simple linear regression, the regression model is $y = \beta_0 + \beta_1 x + \varepsilon$

Regression equation
The equation that describes how the mean or expected value of the dependent variable is related to the independent variable; in simple linear regression,
$E(y) = \beta_0 + \beta_1 x$



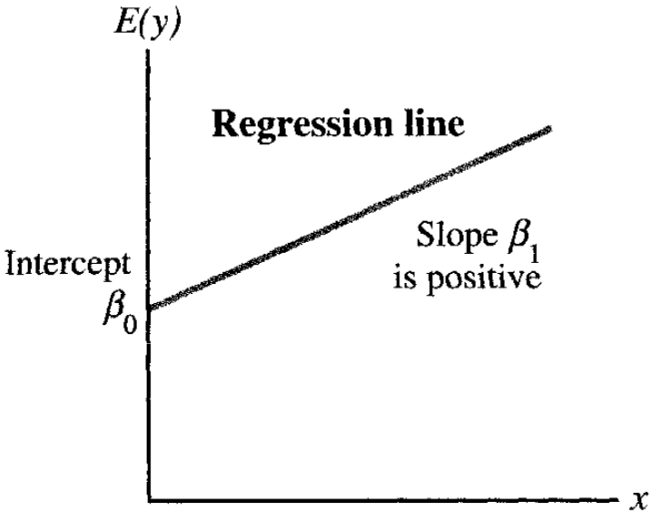
- Model for a simple linear regression:

$$y(x) = \beta_1 x + \beta_0 + \varepsilon$$

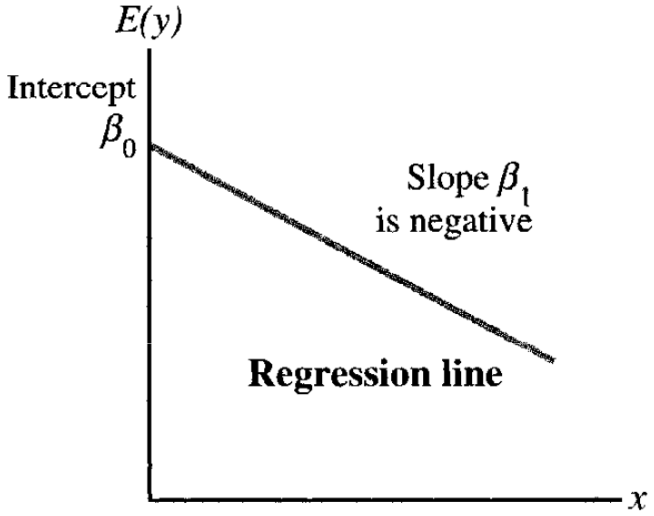
Regression Model and Regression Line

$$y(x) = \beta_1 x + \beta_0 + \varepsilon$$

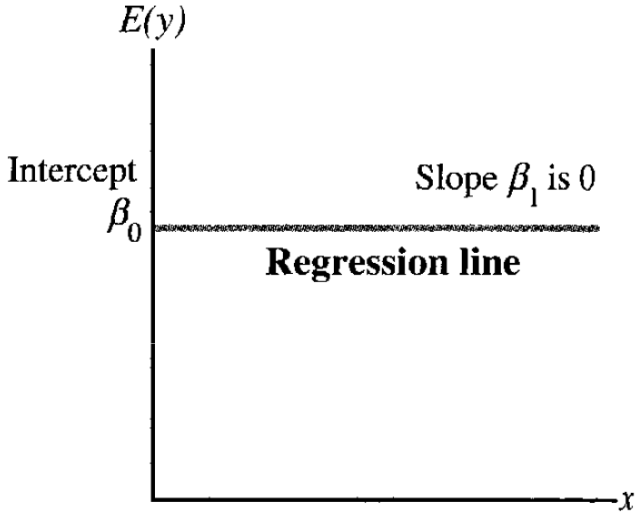
Panel A:
Positive Linear Relationship



Panel B:
Negative Linear Relationship



Panel C:
No Relationship



Estimated regression equation
The estimate of the regression equation developed from sample data by using the least squares method. For simple linear regression, the estimated regression equation is $y = b_0 + b_1 x$

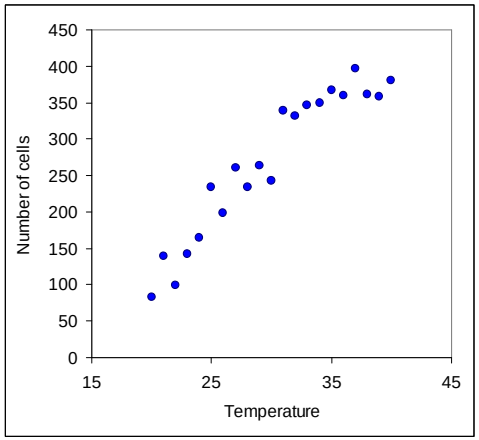
$$y(x) = \beta_1 x + \beta_0 + \varepsilon$$

$$\hat{y}(x) = b_1 x + b_0$$

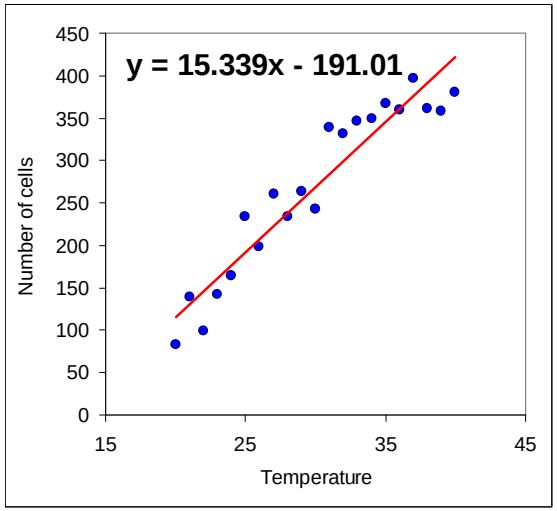
$$E[y(x)] = b_1 x + b_0$$

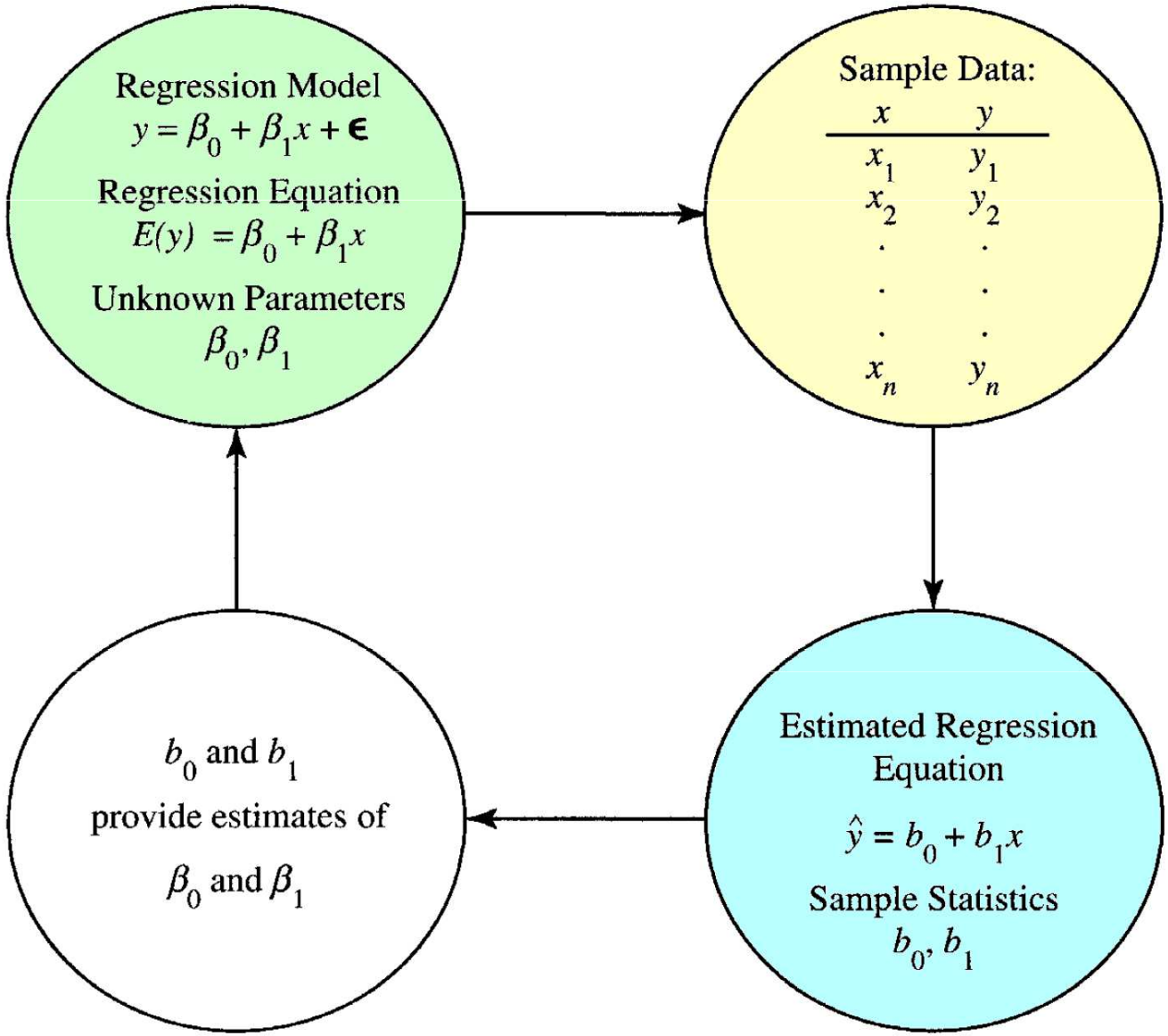
cells.xls

1. Make a scatter plot for the data.



2. Right click to "Add Trendline". Show equation.




Regression Model
$y = \beta_0 + \beta_1 x + \epsilon$
Regression Equation
$E(y) = \beta_0 + \beta_1 x$
Unknown Parameters
β_0, β_1
Sample Data:
x y
x_1 y_1
x_2 y_2
x_n y_n
Estimated Regression Equation
$\hat{y} = b_0 + b_1 x$
Sample Statistics
b_0, b_1
b_0 and b_1
provide estimates of
β_0 and β_1

Least squares method

A procedure used to develop the estimated regression equation.

The objective is to minimize $\sum(y_i - \hat{y}_i)^2$

y_i = observed value of the dependent variable for the ith observation

$\hat{y}_i$ = estimated value of the dependent variable for the ith observation

Slope: $$b_1 = \frac{\sum(x_i - m_x)(y_i - m_y)}{(x_1 - m_x)^2}$$

Intersect: $$b_0 = m_y - b_1 m_x$$

Coefficient of Determination

Sum squares due to **error**

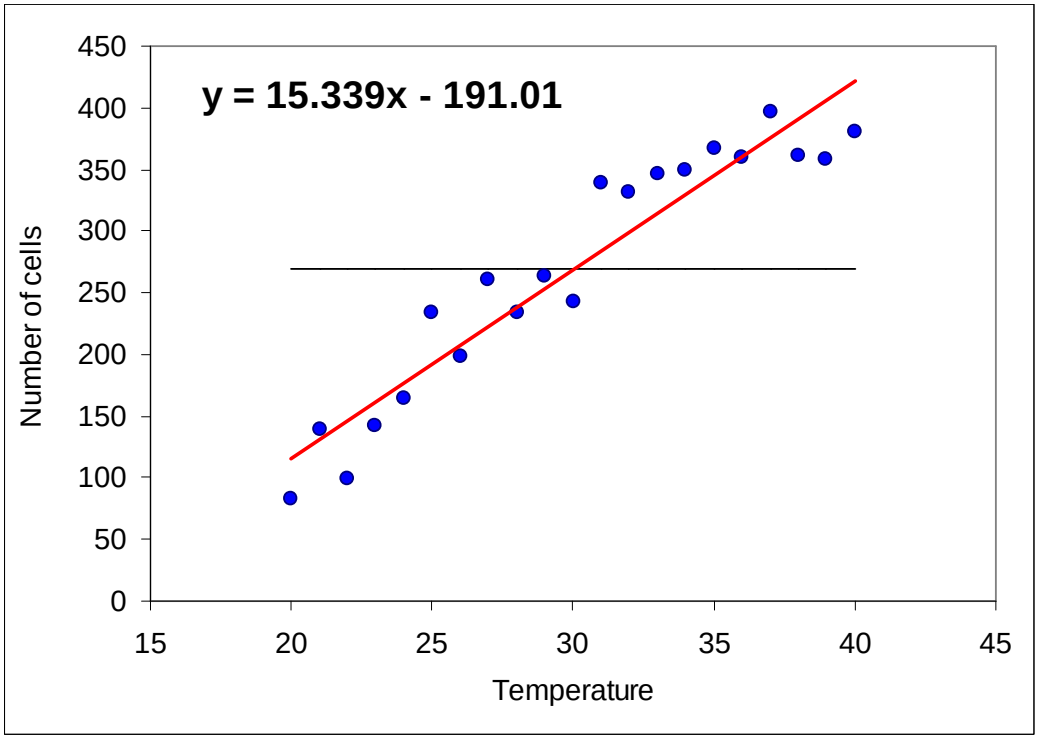
$$SSE = \sum (y_i - \hat{y}_i)^2$$

Sum squares total

$$SST = \sum (y_i - \bar{y})^2$$

Sum squares due to regression

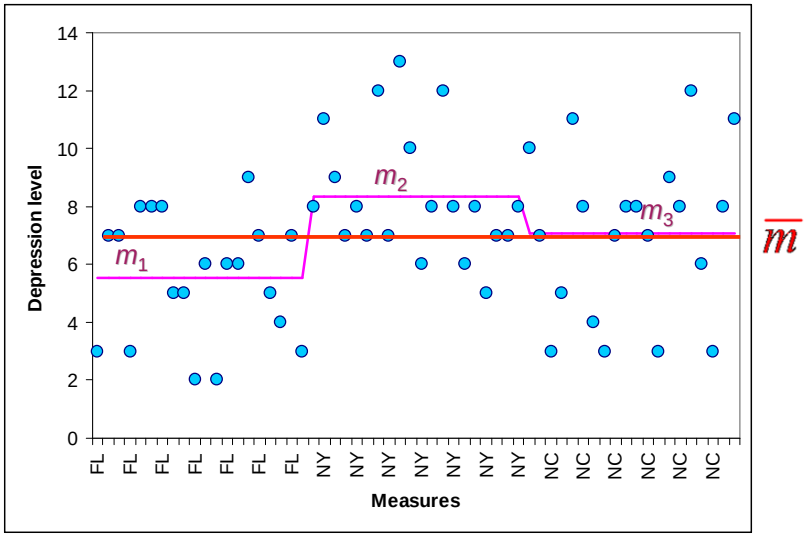
$$SSR = \sum (\hat{y}_i - \bar{y})^2$$



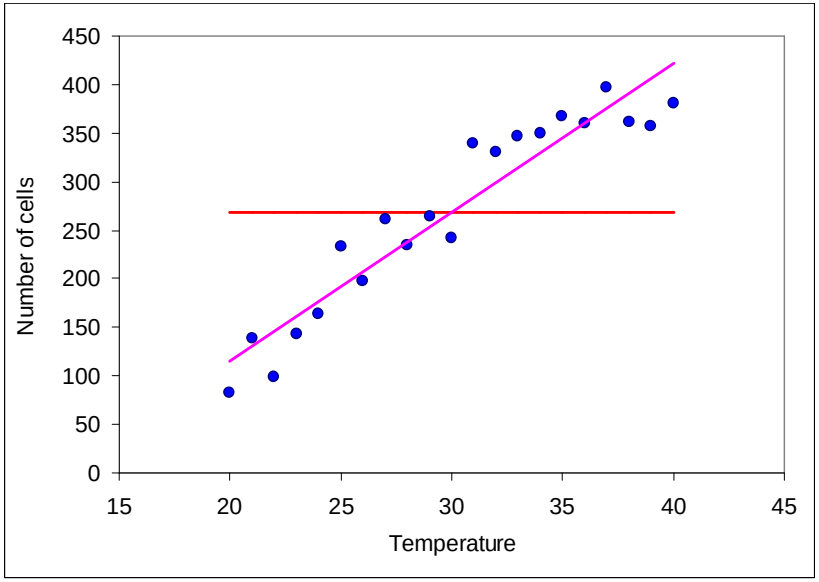
The Main Equation

$$SST = SSR + SSE$$

ANOVA and Regression: Testing for Significance



$$SST = SSTR + SSE$$



$$SST = SSR + SSE$$

H_0: $\beta_1 = 0$ *insignificant*

H_a: $\beta_1 \neq 0$

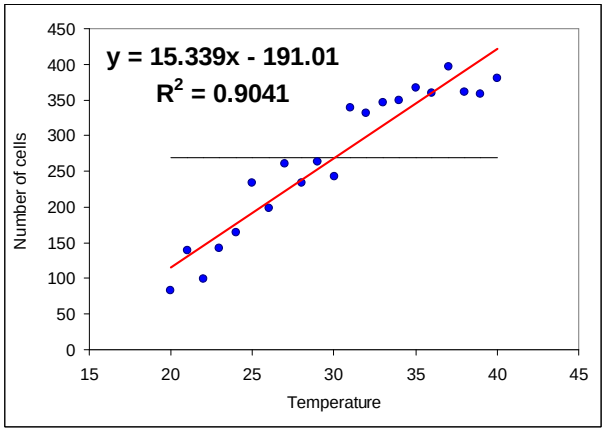
Coefficient of Determination

$$SSE = \sum (y_i - \hat{y}_i)^2$$

$$SST = \sum (y_i - \bar{y})^2$$

$$SSR = \sum (\hat{y}_i - \bar{y})^2$$

$$SST = SSR + SSE$$



Coefficient of determination

A measure of the goodness of fit of the estimated regression equation. It can be interpreted as the proportion of the variability in the dependent variable *y* that is explained by the estimated regression equation.

$$R^2 = \frac{SSR}{SST}$$

Correlation coefficient

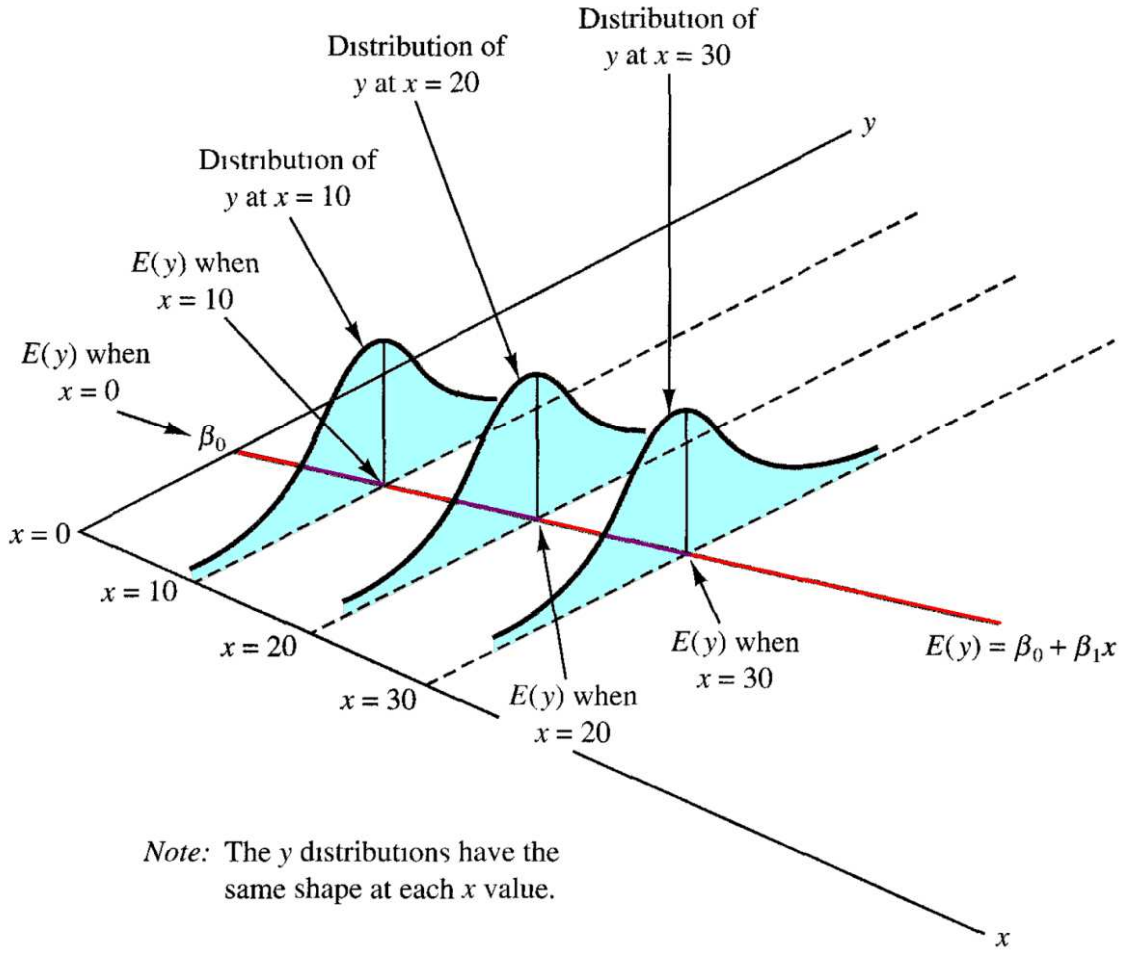
A measure of the strength of the linear relationship between two variables (previously discussed in Lecture 1).

$$r = \text{sign}(b_1)\sqrt{R^2}$$

Assumptions for Simple Linear Regression

1. The error term ε is a random variable with 0 mean, i.e. E[ε]=0
2. The variance of ε, denoted by σ^2, is the same for all values of x
3. The values of ε are independent
3. The term ε is a normally distributed variable

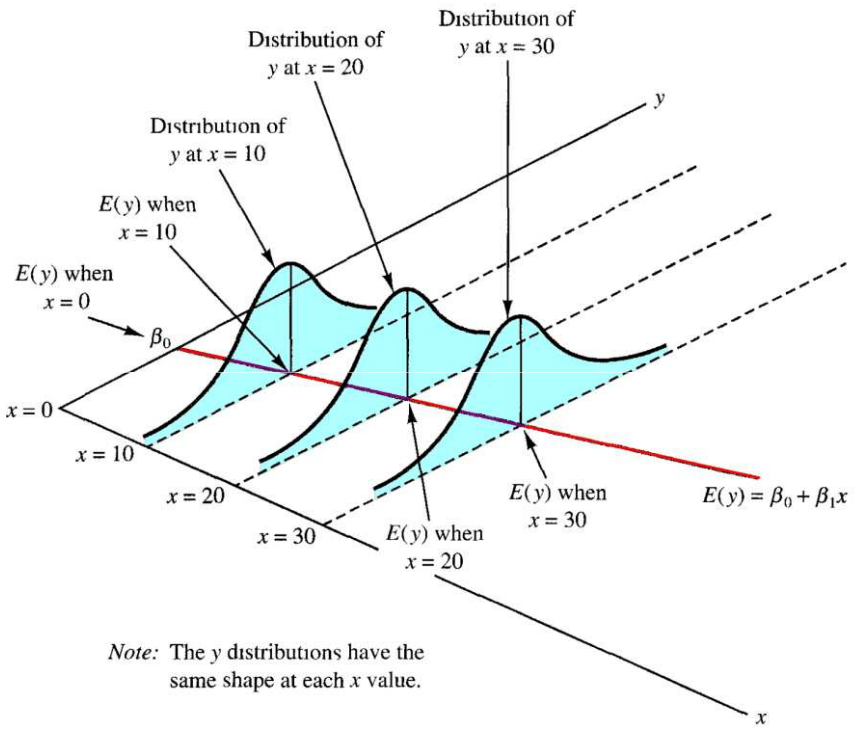
$$y(x) = \beta_1 x + \beta_0 + \varepsilon$$

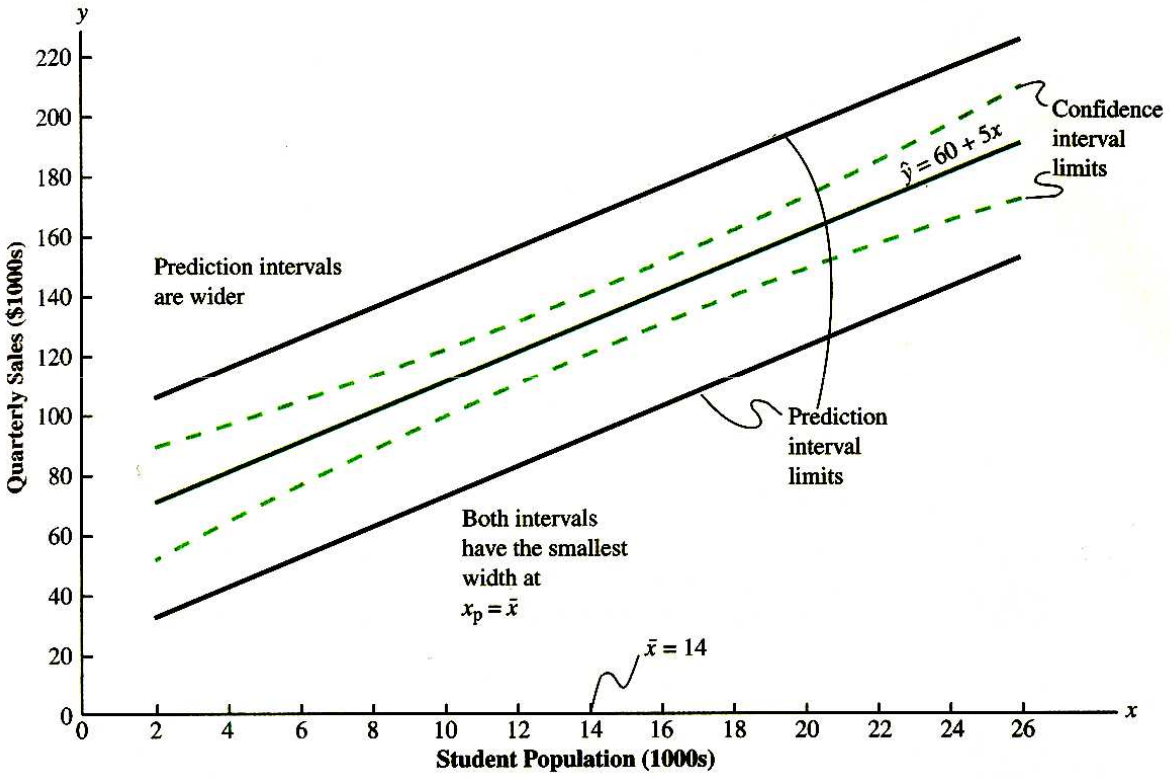


Note: The y distributions have the same shape at each x value.

Confidence interval
The interval estimate of the mean value of y for a given value of x.

Prediction interval
The interval estimate of an individual value of y for a given value of x.

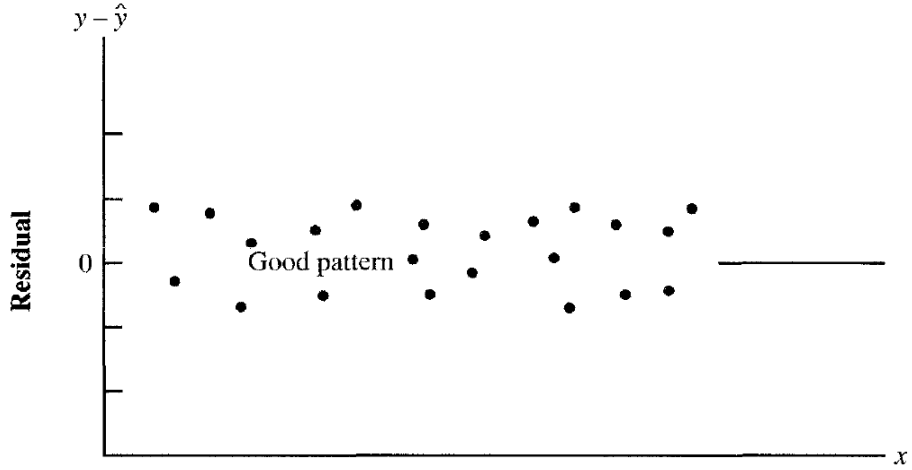




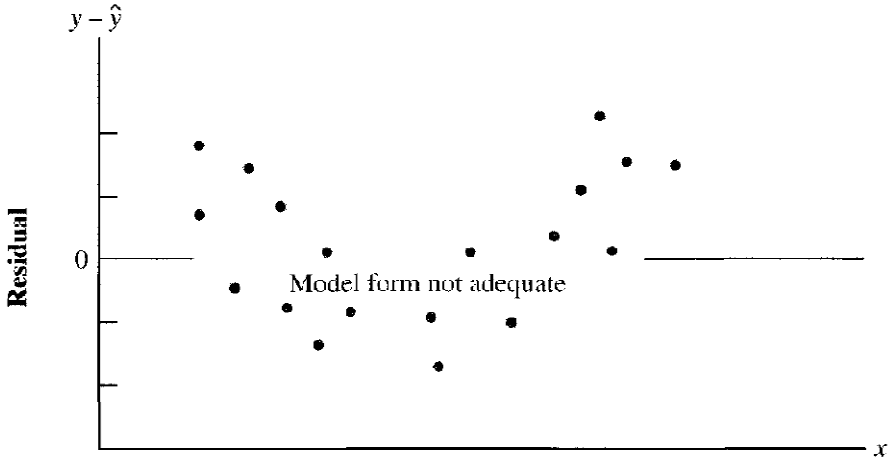
REGRESSION ANALYSIS

Residuals

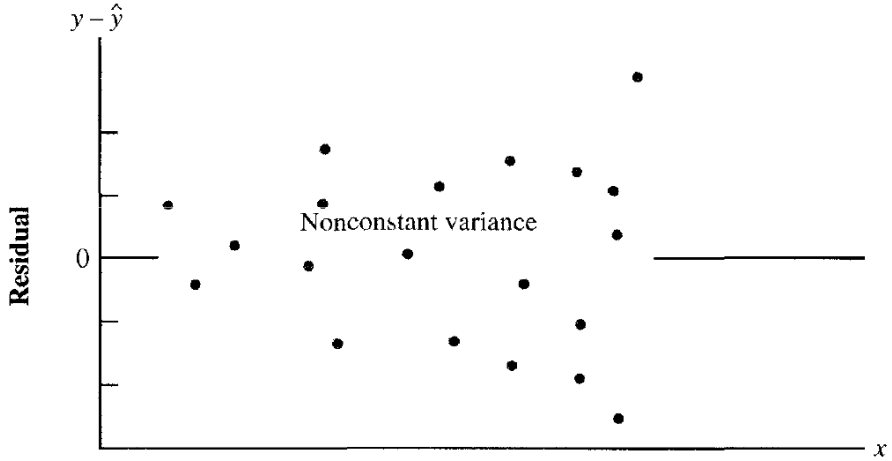
Panel A



Panel C



Panel B



TESTING FOR SIGNIFICANCE

Sampling Distribution for b_1

If assumptions for ε are fulfilled, then the sampling distribution for b_1 is as follows:

$$y(x) = \beta_1 x + \beta_0 + \varepsilon$$

$$\hat{y}(x) = b_1 x + b_0$$

Expected value $E[b_1] = \beta_1$

Variance $\sigma_{b_1} = \dfrac{\sigma}{\sqrt{\sum (x_i - m_x)^2}}$ **= Standard Error**

Distribution: ***normal***

Interval Estimation for β_1

$$\beta_1 = b_1 \pm t_{\alpha/2}^{(n-2)} \frac{\sigma}{\sqrt{\sum (x_i - m_x)^2}}$$

$$\beta_1 = b_1 \pm t_{\alpha/2}^{(n-2)} SE$$

H_0: $\beta_1 = 0$ *insignificant*

H_a: $\beta_1 \neq 0$

1. Build a t-test statistics.

$$t = \frac{b_1}{\sigma_{b_1}} = \frac{b_1}{s}\sqrt{\sum (x_i - m_x)^2}$$

2. Calculate p-value for *t*

1. Build a F-test statistics.

$$F = \frac{MSR}{MSE}$$

$$\text{MSR} = \frac{\text{SSR}}{\text{Number of independent variables}}$$

2. Calculate a p-value

p-value approach: Reject H_0 if *p*-value $\leq \alpha$

Critical value approach: Reject H_0 if $t \leq -t_{\alpha/2}$ or if $t \geq t_{\alpha/2}$

where $t_{\alpha/2}$ is based on a *t* distribution with $n - 2$ degrees of freedom.

Example

cells.xls

1. Calculate manually b_1 and b_0

Intercept	b0=	-191.008119
Slope	b1=	15.3385723

In Excel use the function:

- **= INTERCEPT(y,x)**
- **= SLOPE(y,x)**

2. Let's do it automatically Tools → Data Analysis → Regression

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.950842308
R Square	0.904101095
Adjusted R Square	0.899053784
Standard Error	31.80180903
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	181159.2853	181159.3	179.1253	4.01609E-11
Residual	19	19215.7461	1011.355		
Total	20	200375.0314			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-191.0081194	35.07510626	-5.445689	2.97E-05	-264.4211603	-117.5950784	-264.4211603	-117.5950784
X Variable 1	15.33857226	1.146057646	13.38377	4.02E-11	12.93984605	17.73729848	12.93984605	17.73729848

Example2

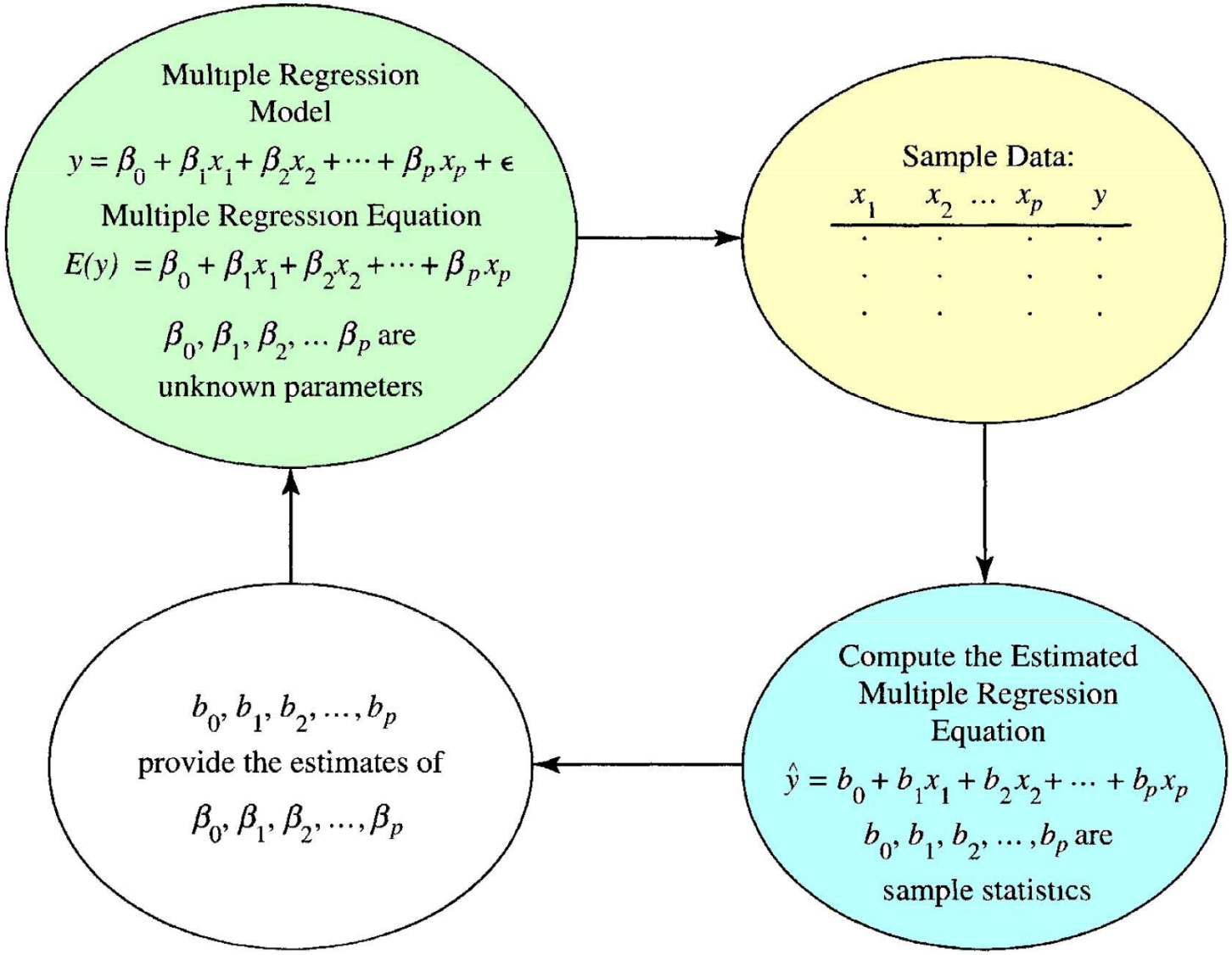
rana.txt

A biology student wishes to determine the relationship between temperature and heart rate in leopard frog, *Rana pipiens*. He manipulates the temperature in 2° increment ranging from 2 to 18°C and records the heart rate at each interval. His data are presented in table rana.txt

1) Build the model and provide the p-value for linear dependency

2) Provide interval estimation for the slope of the dependency

3) Estmate 95% prediction interval for heart rate at 15°


Multiple Regression Model
$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p + \epsilon$
Multiple Regression Equation
$E(y) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p$
$\beta_0, \beta_1, \beta_2, \ldots \beta_p$ are unknown parameters
Sample Data:
x_1 x_2 ... x_p y
Compute the Estimated Multiple Regression Equation
$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + \cdots + b_p x_p$
$b_0, b_1, b_2, \ldots, b_p$ are sample statistics
$b_0, b_1, b_2, \ldots, b_p$ provide the estimates of $\beta_0, \beta_1, \beta_2, \ldots, \beta_p$

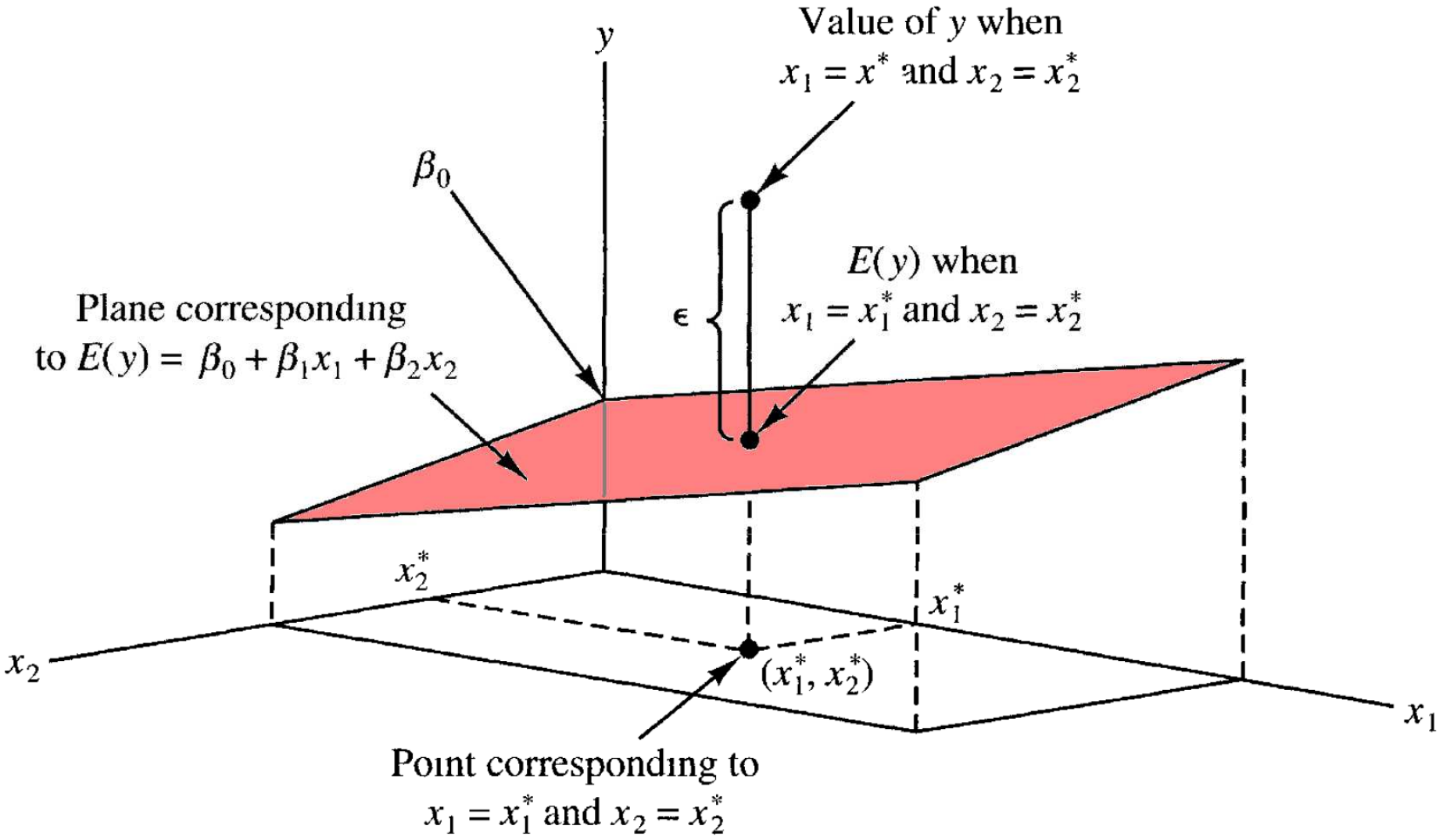
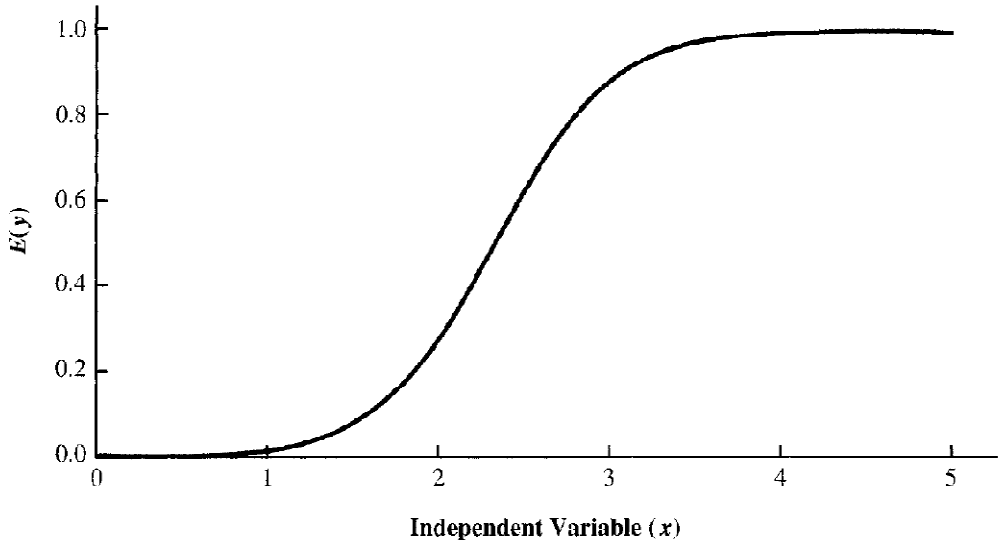

y
Value of y when
$x_1 = x^*$ and $x_2 = x_2^*$
β_0
$E(y)$ when
$x_1 = x_1^*$ and $x_2 = x_2^*$
ϵ
Plane corresponding
to $E(y) = \beta_0 + \beta_1 x_1 + \beta_2 x_2$
x_2^*
x_1^*
x_2
(x_1^*, x_2^*)
x_1
Point corresponding to
$x_1 = x_1^*$ and $x_2 = x_2^*$

FIGURE 15.12 LOGISTIC REGRESSION EQUATION FOR $\beta_0 = -7$ AND $\beta_1 = 3$



$$E(y) = P(y = 1 \mid x_1, x_2, \ldots, x_p) = \frac{\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p)}{1 + \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p)}$$

Correlation Analysis of Transcriptomic Data

Gene regulatory networks (GRN) in living cells can be considered as extremely complex information processing systems. Despite their complexity, the main feature of the GRN is their robustness and ability to form a proper biochemical respond to a wide range of extracellular conditions. The knowledge about the part of GRN related to a specific bio-function of cellular process is of extreme importance for controlling them. Another important aspect of understanding cell functionality is linked to knowledge about the regulatory effect of small non-coding micro-RNA (**miRNA**). miRNAs influence most fundamental biological processes by ultimately altering the expression levels of proteins either through degradation of **mRNA** or through interference with mRNA translation. miRNAs tend to have long half lives and therefore represent promising candidates to be used as disease markers and therapeutic targets.

Being a reverse-engineering task, the GRN reconstruction is highly challenging, and requires analysis of large sets of experimental data. One of the straightest ways to reconstruct GRN is based on co-expression (CE) analysis of transcriptomic data from cDNA microarrays. Two significantly co-expressed genes or a gene and miRNA have the same or inverted expression profile over a number of samples. Biologically this is a good evidence for either a direct interaction between the genes or their mutual participation in the same biological function.

The performance of the software was tested using public mRNA and miRNA expression data from 14 various cell lines (A498, ACHN, CAKI1, CCRFCEM, HCT15, HL60, K562, MALME3M, MCF7, MOLT4, NCIH226, NCIH522, RPMI8226, SKOV3). Data from 42 Affymetrix® HGU133plus2 arrays and 14 miRNA custom microarray experiments were downloaded from public repositories (ref. E-MTAB-37 and E-MEXP-1029, http://www.ebi.ac.uk) , normalized and analyzed.

Tool: http://edu.sablab.net/biostat2/coexpress.zip

Data: http://edu.sablab.net/biostat2/data-mir-mrna_14cl.zip

Thank you for your attention

to be continued…