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Molecular Biology

# Linear Modelling: Simple Regression

12<sup>th</sup> of March 2021

R. Nicholls / D.-L. Couturier / M. Fernandes

# **Introduction:**

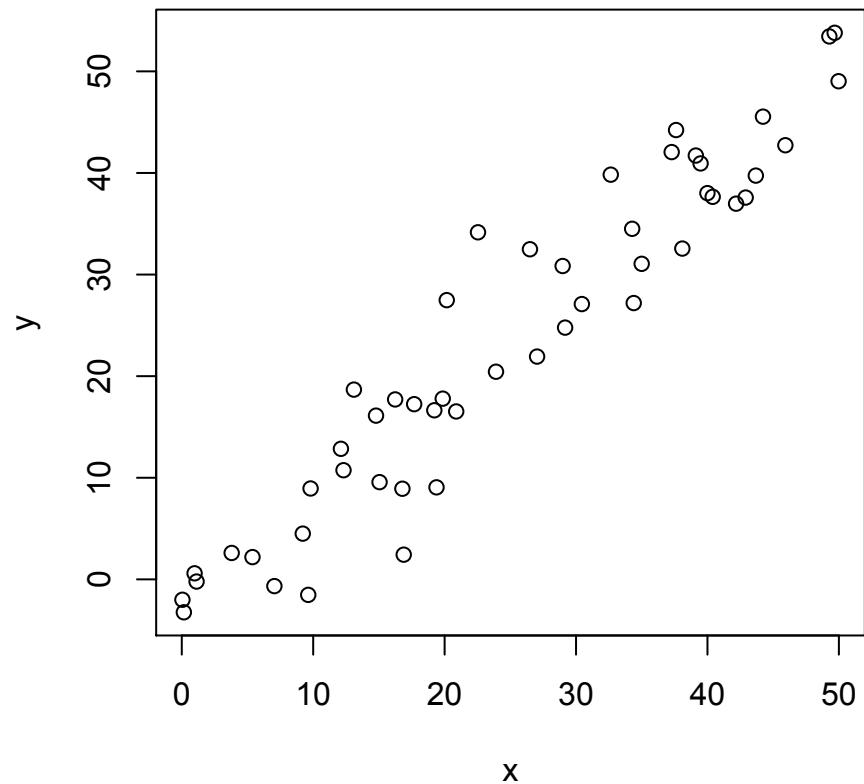
## **ANOVA**

- Used for testing hypotheses regarding differences between groups
- Considers the variation within and between groups

## **Regression**

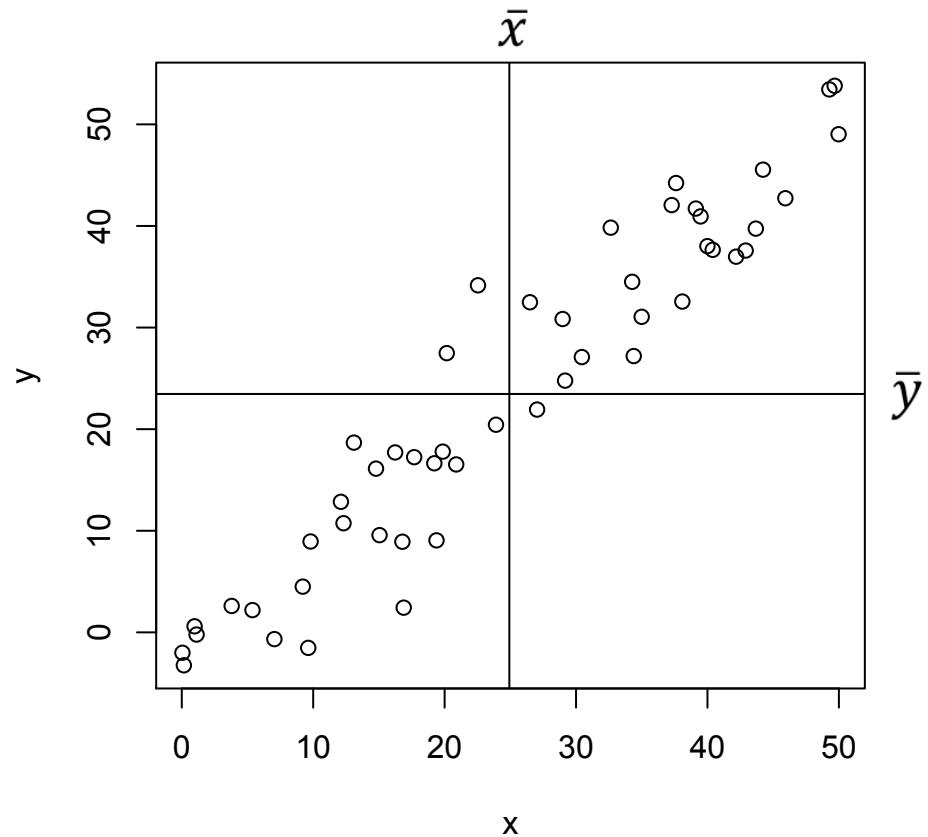
- Used for revealing and investigating relationships between input and output variables
- Aim is to model data, and extrapolate as much information as possible

# Correlation:

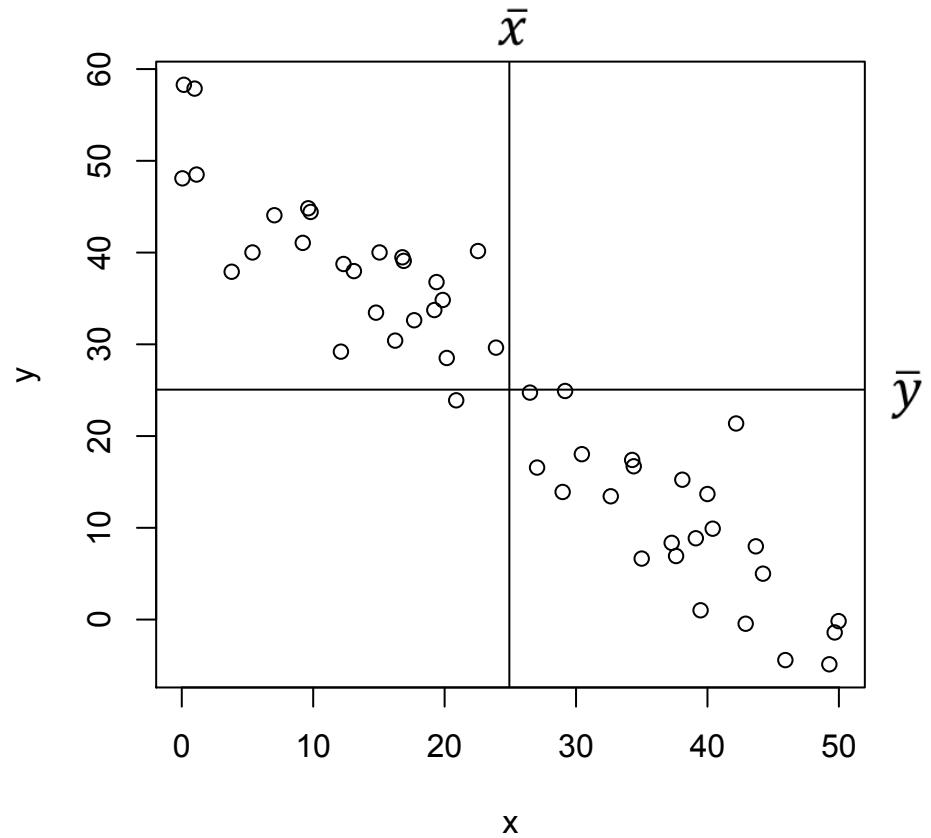


How to measure the strength of a linear relationship between variables?

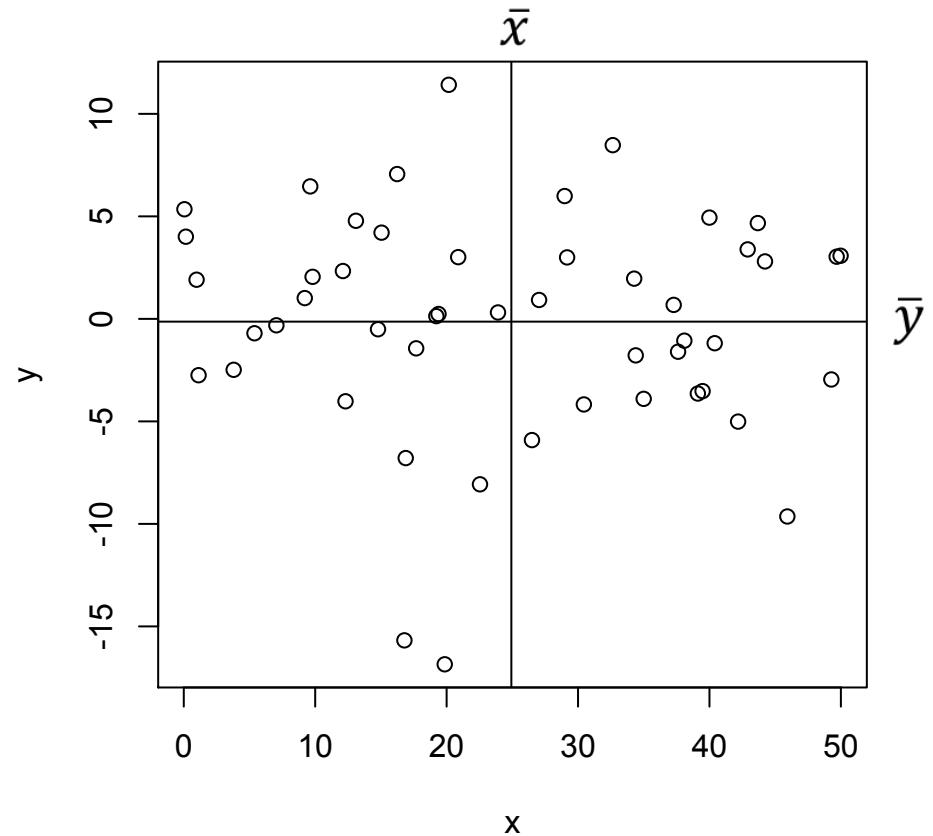
# Correlation:



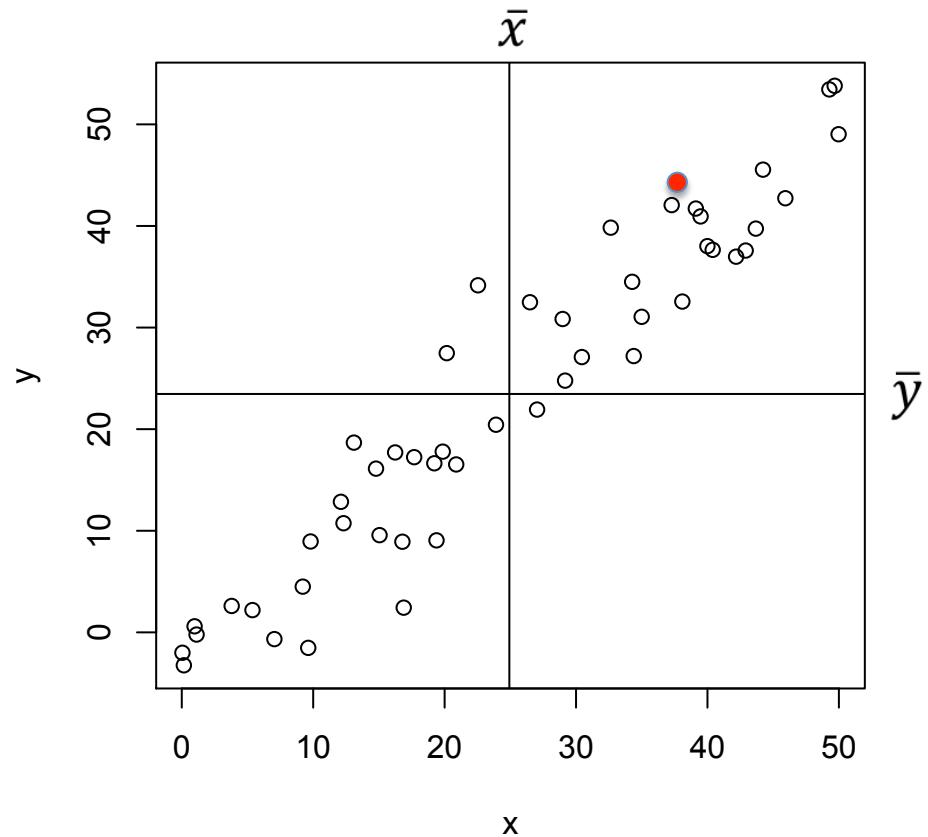
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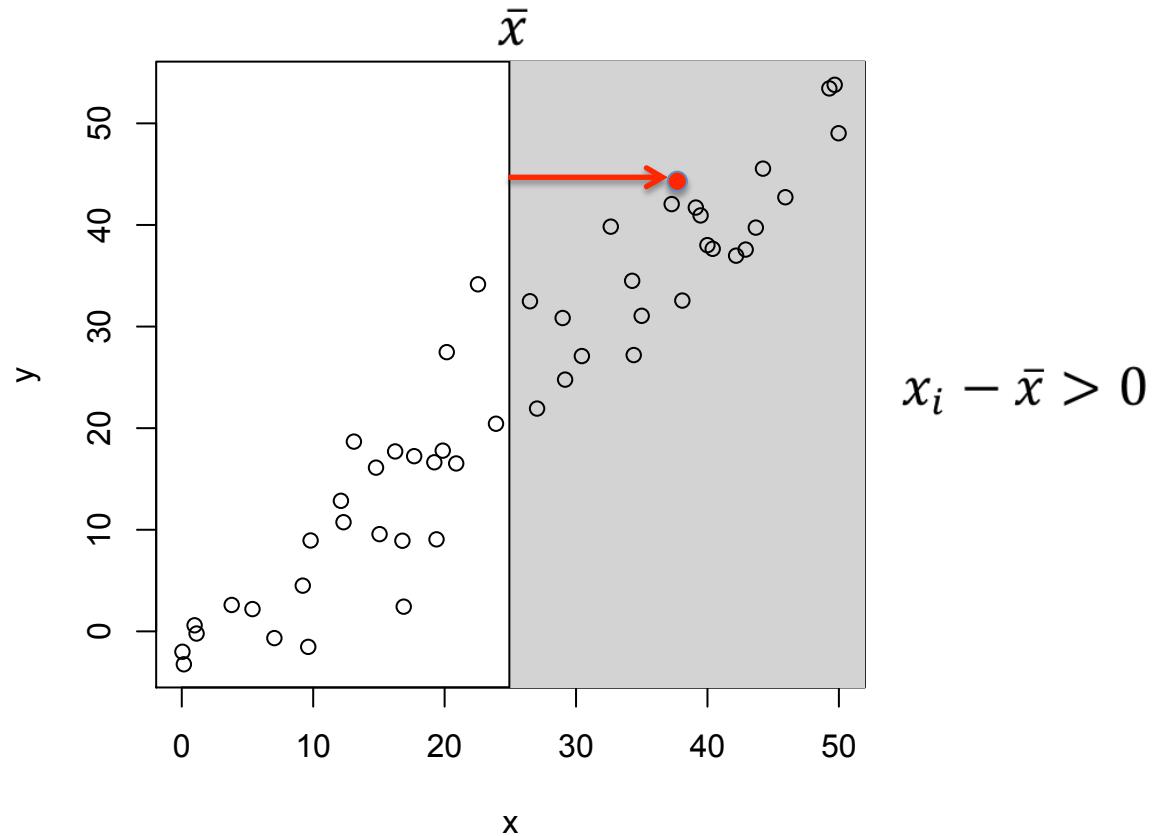
# Correlation:



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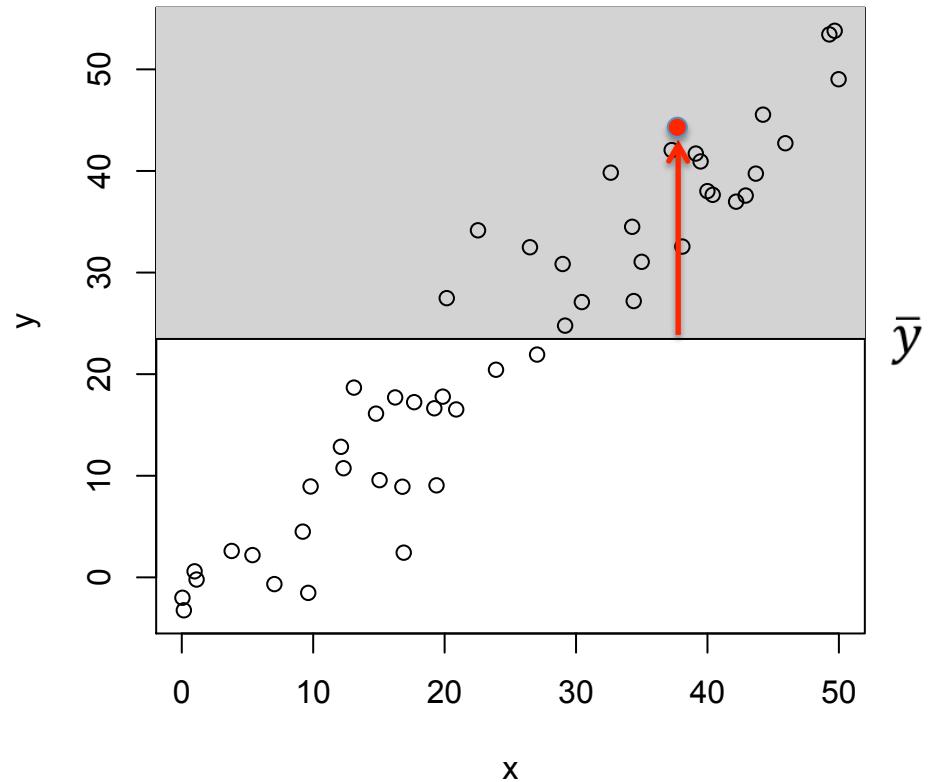


# Correlation:

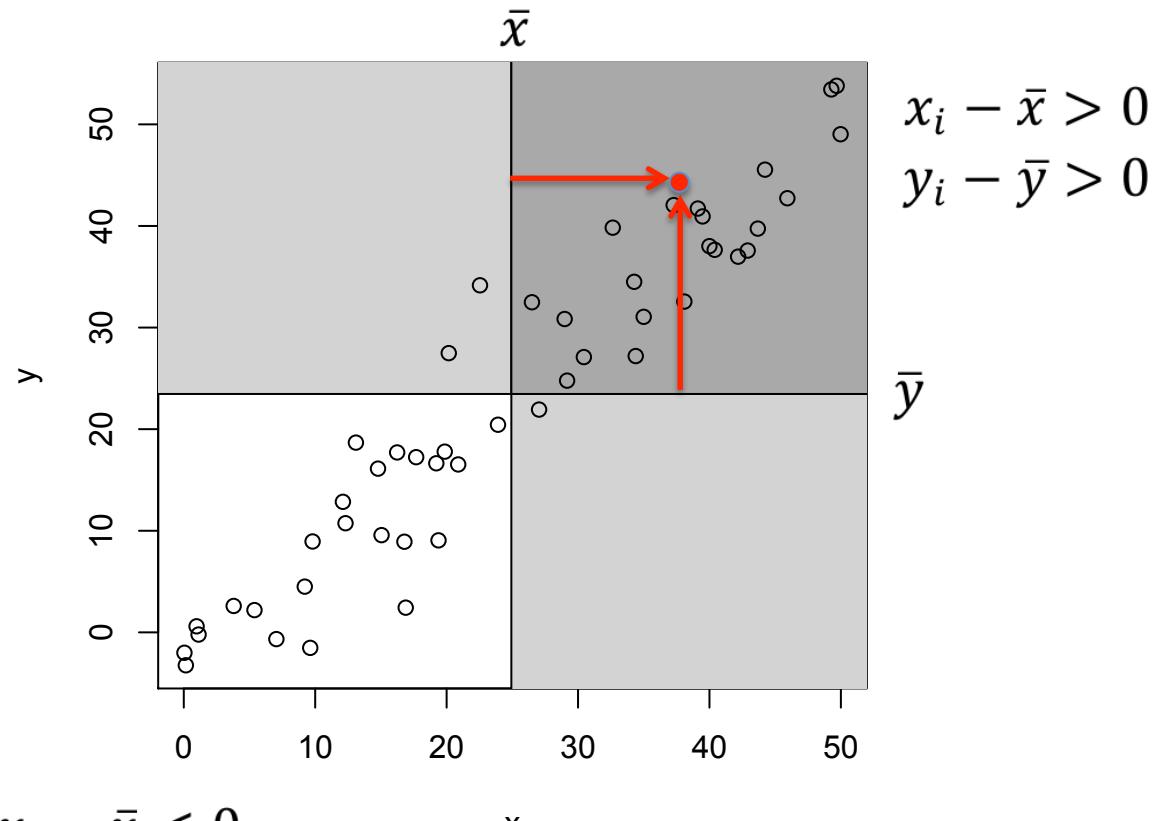


# Correlation:

$$y_i - \bar{y} > 0$$



# Correlation:

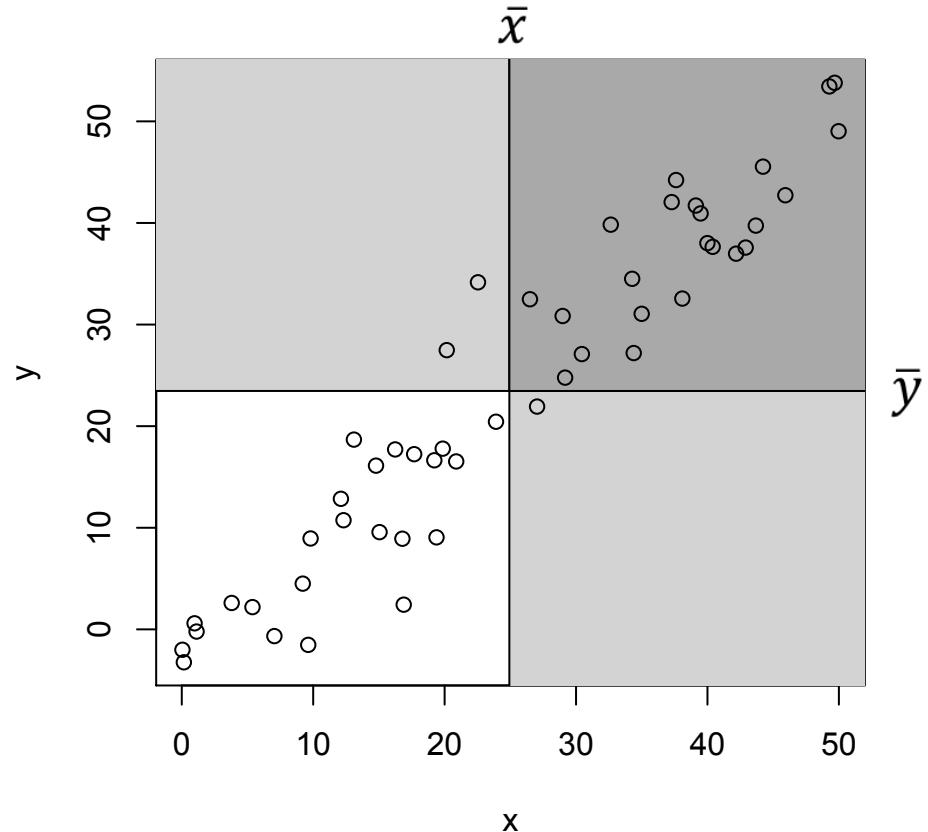


$$x_i - \bar{x} < 0$$

$$y_i - \bar{y} < 0$$

# Correlation:

$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$

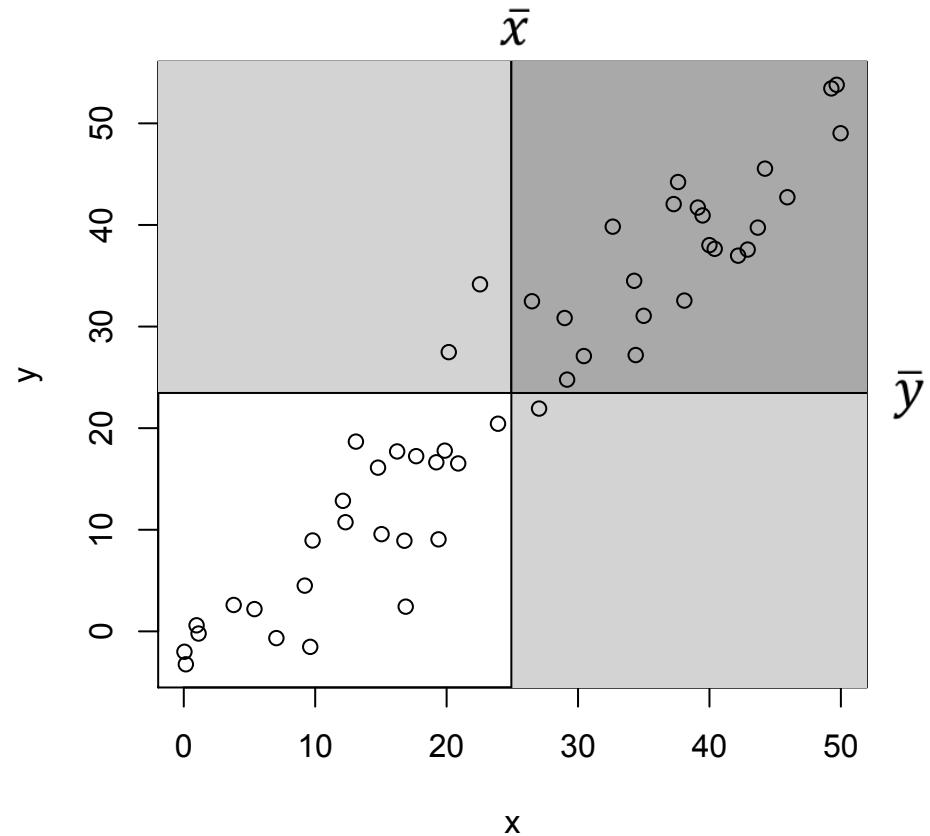


$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$

# Correlation:

$$(x_i - \bar{x})(y_i - \bar{y}) < 0$$

$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$



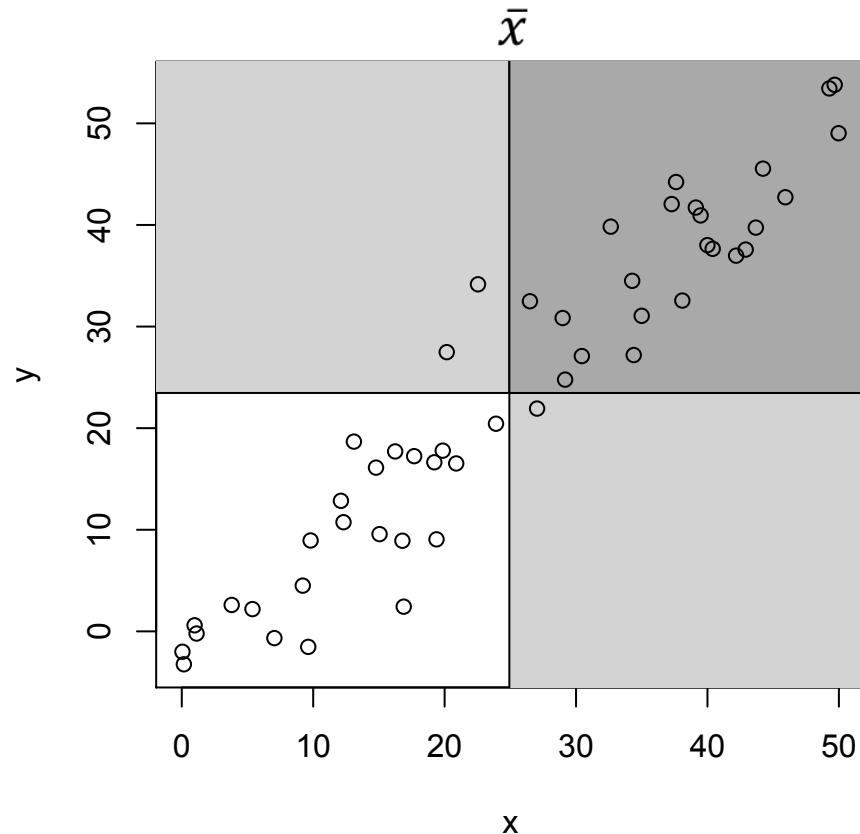
$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$

$$(x_i - \bar{x})(y_i - \bar{y}) < 0$$

# Correlation:

$$(x_i - \bar{x})(y_i - \bar{y}) < 0$$

$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$



$$(x_i - \bar{x})(y_i - \bar{y}) > 0$$

$$(x_i - \bar{x})(y_i - \bar{y}) < 0$$

Positively correlated:

$$\sum_i (x_i - \bar{x})(y_i - \bar{y}) \gg 0$$

Negatively correlated:

$$\sum_i (x_i - \bar{x})(y_i - \bar{y}) \ll 0$$

Uncorrelated:

$$\sum_i (x_i - \bar{x})(y_i - \bar{y}) \approx 0$$

# Correlation:

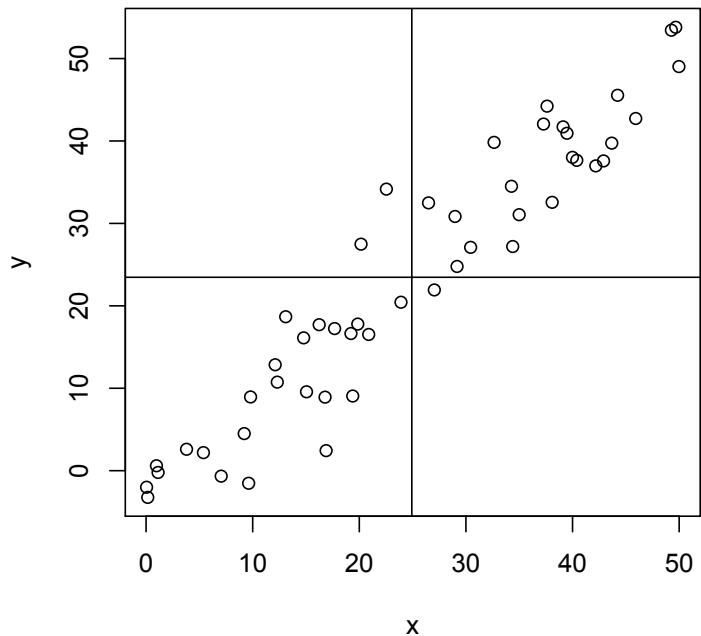
Pearson's product-moment correlation coefficient:

$$r_{X,Y} = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}}$$

Coefficient of Variation ( $R^2$  value):

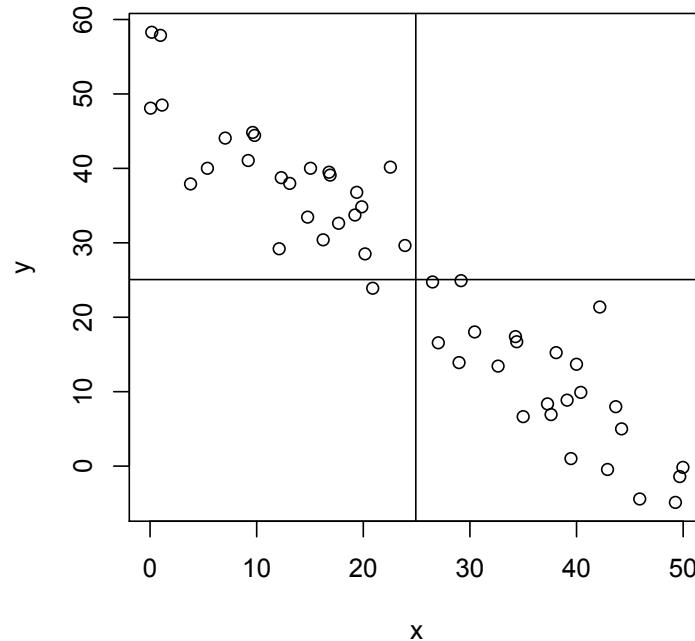
$$R_{X,Y}^2 = r_{X,Y}^2$$

# Correlation:



$$r = 0.931$$

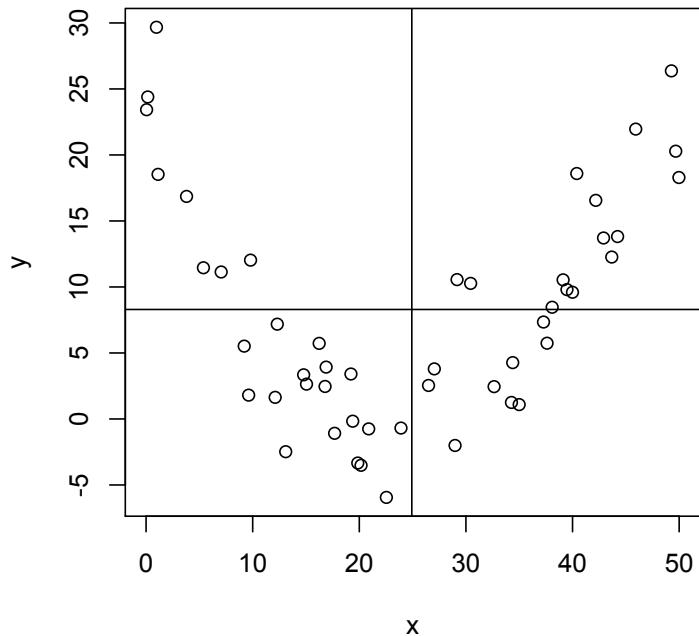
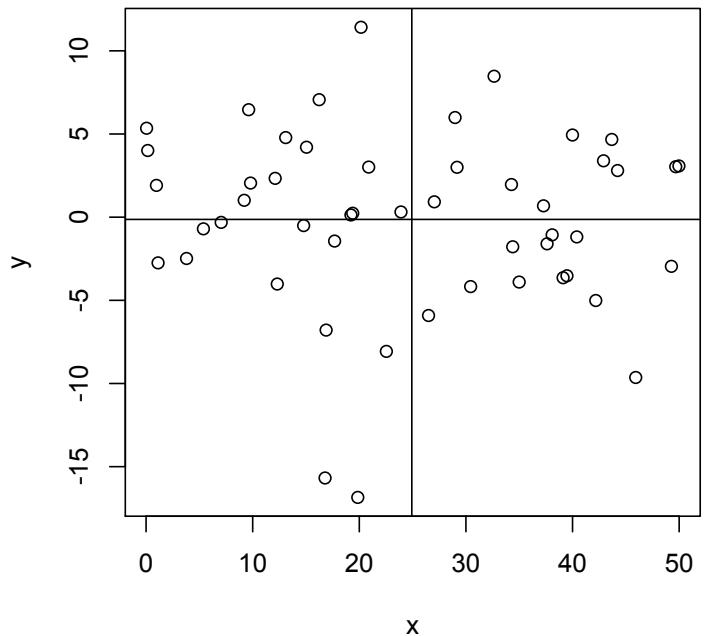
$$R^2 = 0.866$$



$$r = -0.949$$

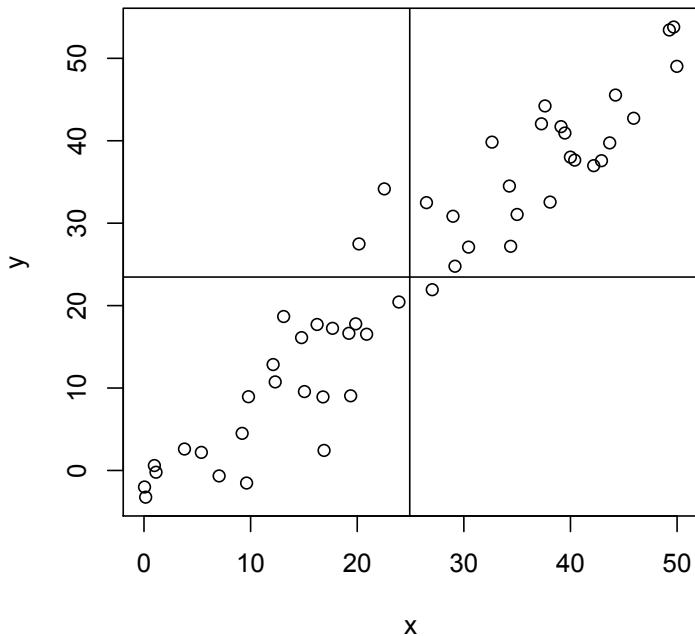
$$R^2 = 0.901$$

# Correlation:

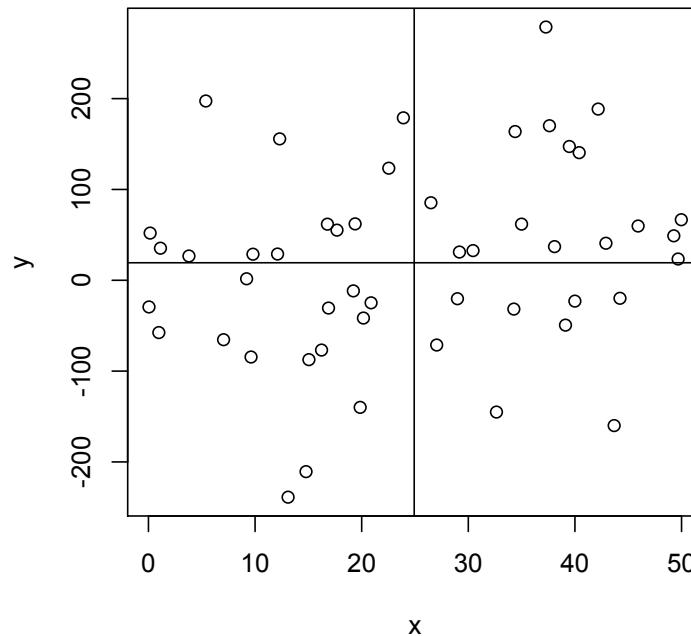


# Correlation:

Can I say whether my data are correlated?  
Is an observed correlation significant?



data: x and y  
 $t = 17.613$ ,  $df = 48$ , p-value < 2.2e-16  
alternative hypothesis: true correlation is not equal to 0  
95 percent confidence interval:  
0.8802556 0.9602168  
sample estimates:  
cor  
0.9305923



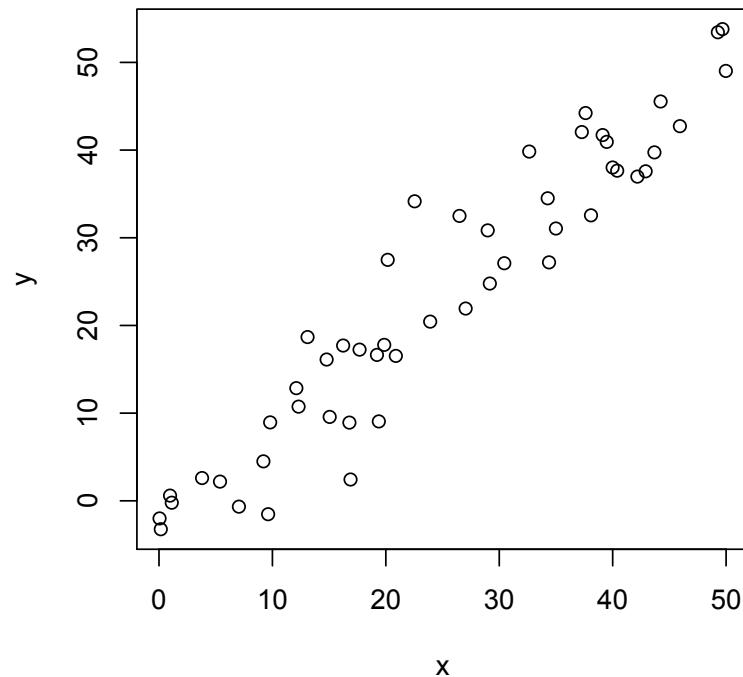
data: x and y  
 $t = 1.5609$ ,  $df = 48$ , p-value = 0.1251  
alternative hypothesis: true correlation is not equal to 0  
95 percent confidence interval:  
-0.06238066 0.46941403  
sample estimates:  
cor  
0.2197833

# Simple Regression:

Aims:

- To investigate linear correlation between two variables in more detail
- Be able to predict a response given knowledge of the independent variable

Response variable  
Dependent variable



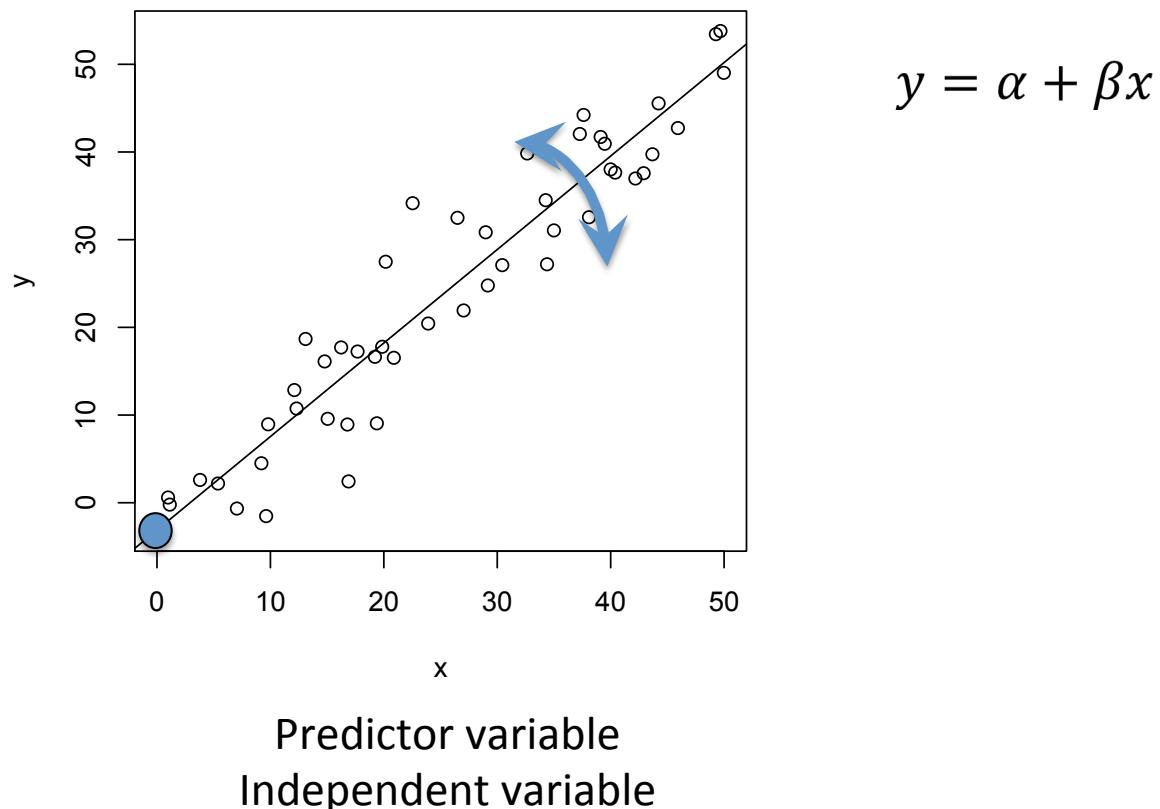
Predictor variable  
Independent variable

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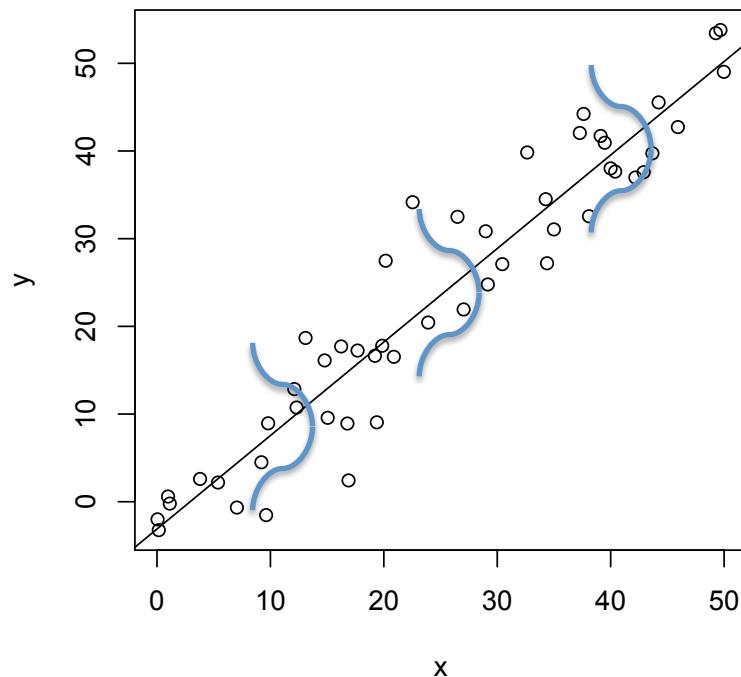


# Simple Regression:

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$$y = \alpha + \beta x + \varepsilon$$

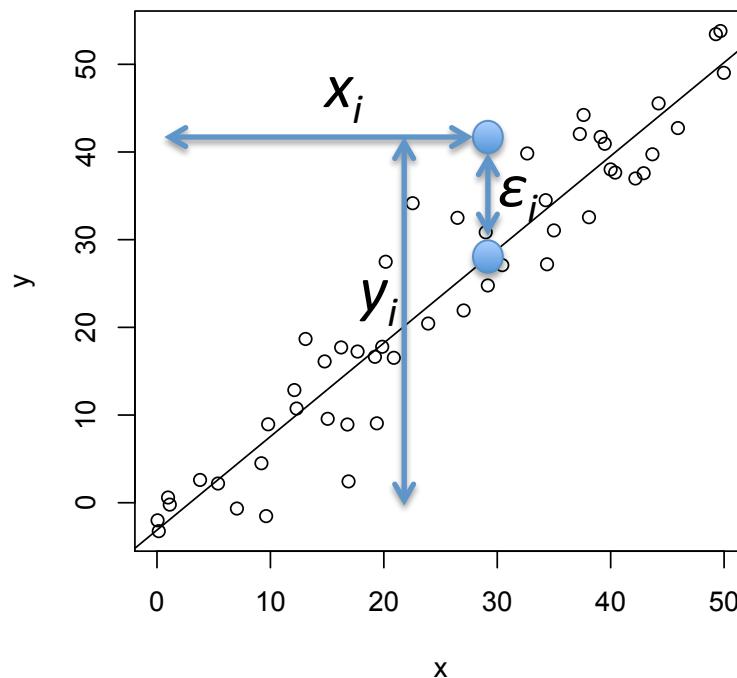
$$\varepsilon \sim N(0, \sigma^2)$$

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Response variable  
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$$y = \alpha + \beta x + \varepsilon$$

$$\varepsilon \sim N(0, \sigma^2)$$

For the  $i^{\text{th}}$  observation:

$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$\varepsilon_i$  = errors, residuals

$$y_i = \alpha + \beta x_i + \varepsilon_i$$

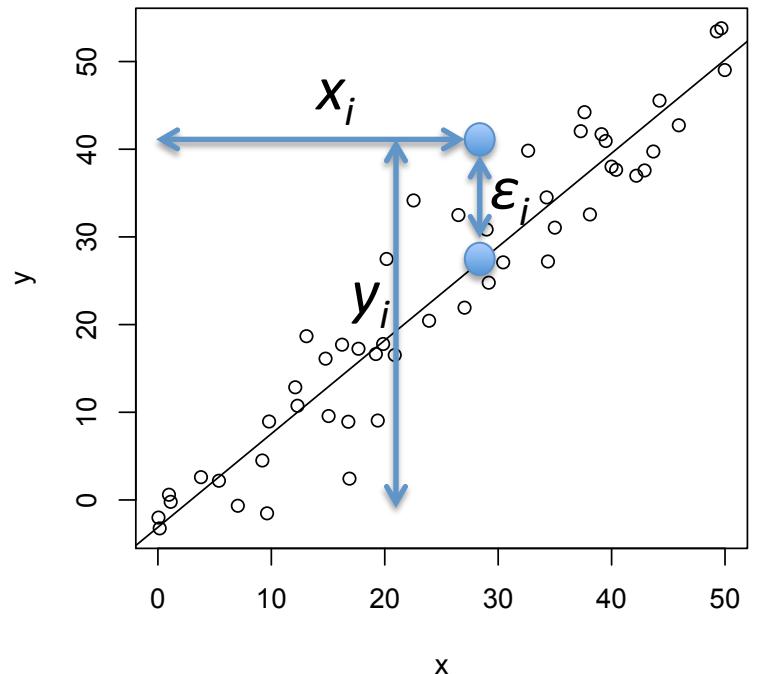
$$\varepsilon_i \sim N(0, \sigma^2)$$

## Simple Regression:

So how do we fit the regression line?

Suppose we know parameter estimates  $\hat{\alpha}$  and  $\hat{\beta}$

Observations:  $y = \alpha + \beta x + \varepsilon$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

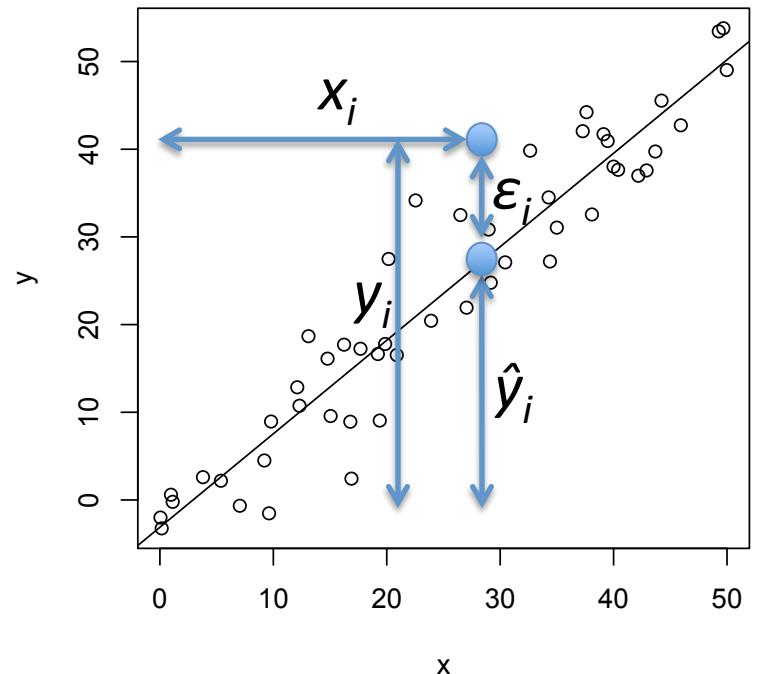
$$\mathbf{y} = \alpha + \beta \mathbf{x} + \boldsymbol{\varepsilon}$$

So how do we fit the regression line?

Suppose we know parameter estimates  $\hat{\alpha}$  and  $\hat{\beta}$

Observations:  $\mathbf{y} = \alpha + \beta \mathbf{x} + \boldsymbol{\varepsilon}$

Fitted values:  $\hat{\mathbf{y}} = E(\mathbf{y} | \mathbf{x}; \hat{\alpha}, \hat{\beta})$   
 $= E(\alpha + \beta \mathbf{x} + \boldsymbol{\varepsilon} | \hat{\alpha}, \hat{\beta})$   
 $= E(\hat{\alpha} + \hat{\beta} \mathbf{x} + \boldsymbol{\varepsilon})$   
 $= \hat{\alpha} + \hat{\beta} \mathbf{x}$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

$$\mathbf{y} = \alpha + \beta \mathbf{x} + \boldsymbol{\varepsilon}$$

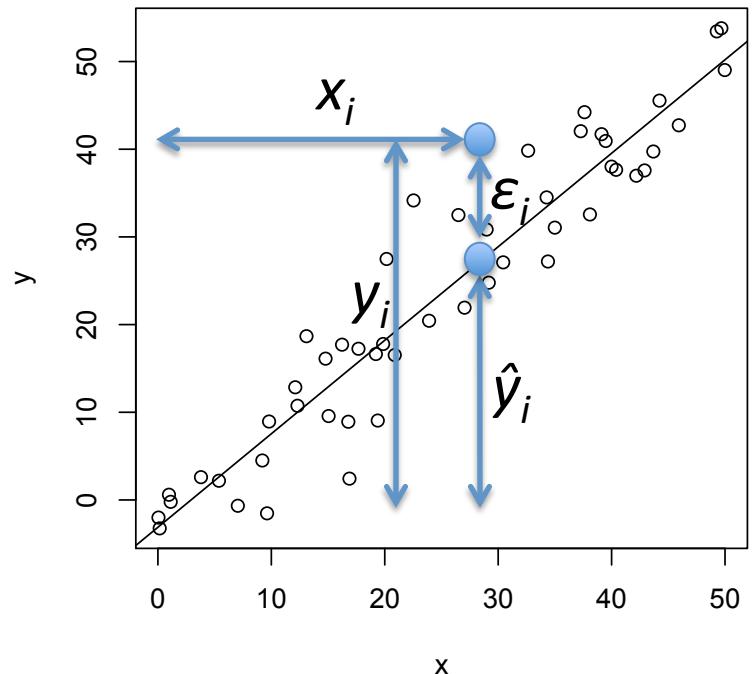
$$\hat{\mathbf{y}} = \hat{\alpha} + \hat{\beta} \mathbf{x}$$

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Residuals:  $\boldsymbol{\varepsilon} = \mathbf{y} - \hat{\mathbf{y}}$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

$$\mathbf{y} = \alpha + \beta \mathbf{x} + \boldsymbol{\varepsilon}$$

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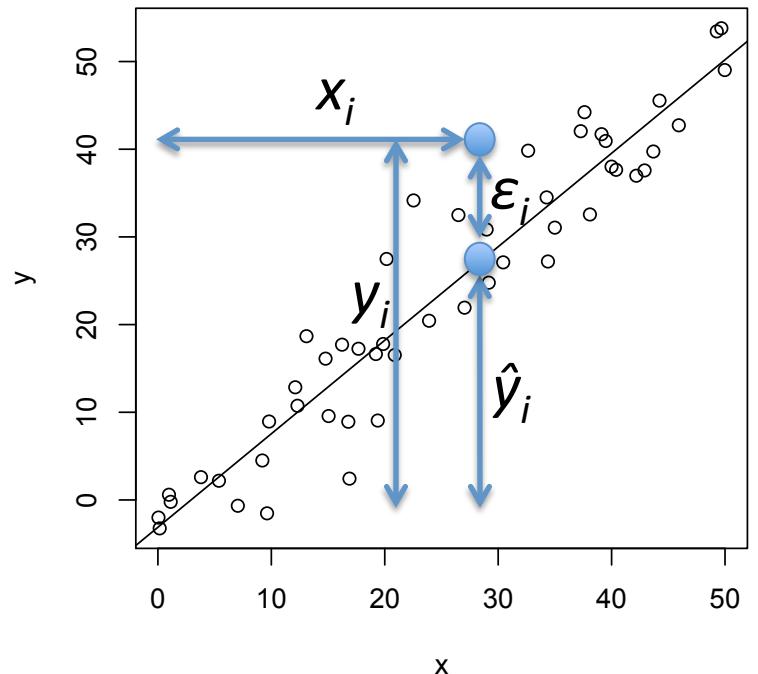
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$$y_i = \alpha + \beta x_i + \varepsilon_i$$

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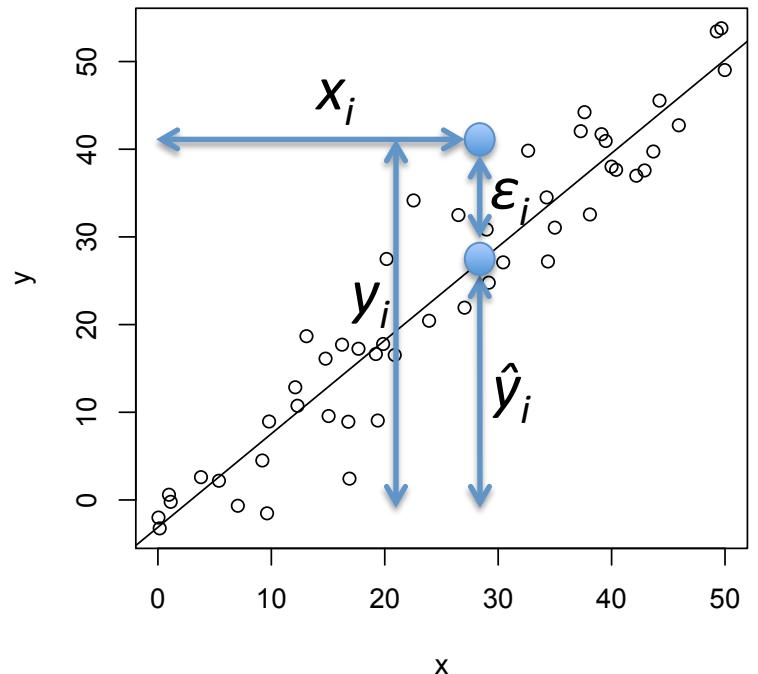
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$$\mathbf{y} \sim N(\hat{\mathbf{y}}, \sigma^2)$$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

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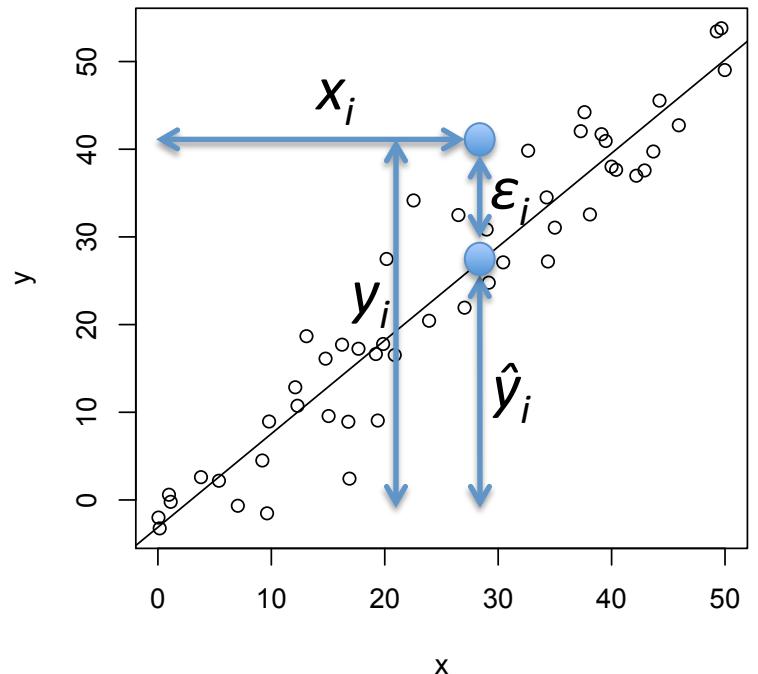
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$$\mathbf{y} \sim N(\hat{\mathbf{y}}, \sigma^2)$$

$$f(y|x; \hat{\alpha}, \hat{\beta}) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(y-\hat{y})^2}{2\sigma^2}}$$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

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## Simple Regression:

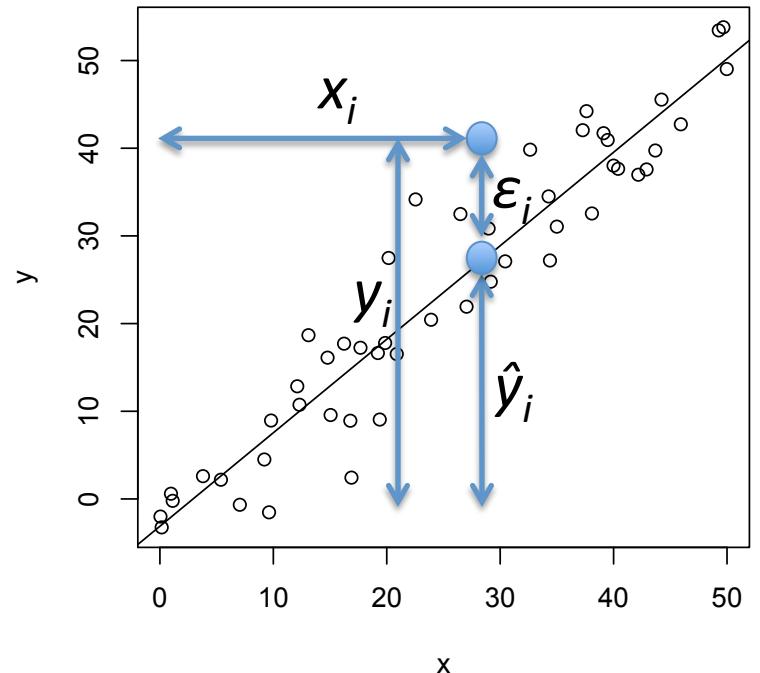
So how do we fit the regression line?

Obtain estimates  $\hat{\alpha}$  and  $\hat{\beta}$

Maximise likelihood of parameters given the data

$$f(\mathbf{y}|\mathbf{x}; \hat{\alpha}, \hat{\beta}) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(\mathbf{y}-\hat{\mathbf{y}})^2}{2\sigma^2}}$$

$$\begin{aligned} \mathcal{L}(\hat{\alpha}, \hat{\beta} | \mathbf{y}, \mathbf{x}) &= \prod_i f(y_i | x_i; \hat{\alpha}, \hat{\beta}) \\ &= \prod_i \frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(y_i - \hat{y}_i)^2}{2\sigma^2}} \end{aligned}$$



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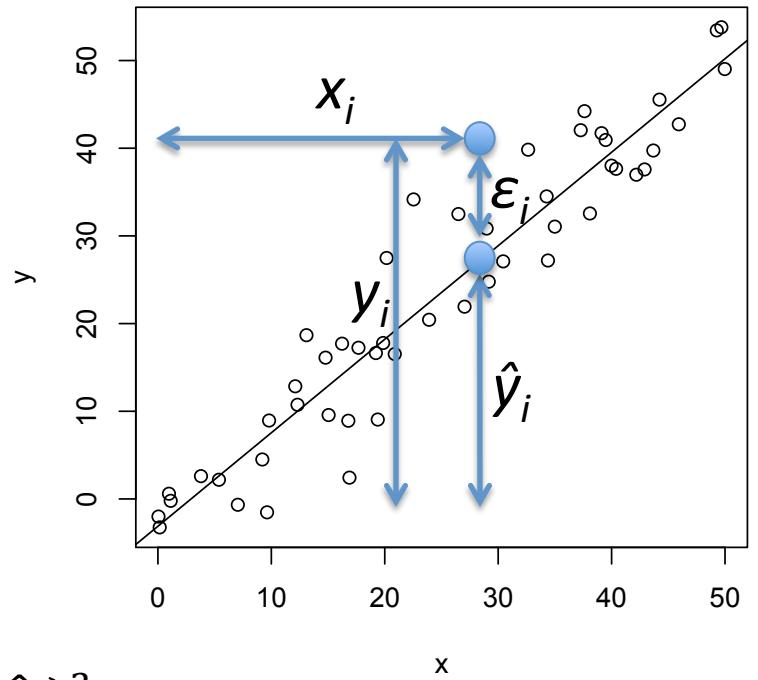
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$$\ln \mathcal{L}(\hat{\alpha}, \hat{\beta} | \mathbf{y}, \mathbf{x}) = \sum_i \left( \frac{-1}{2} \ln(2\pi\sigma^2) - \frac{(y_i - \hat{y}_i)^2}{2\sigma^2} \right)$$

$$= \frac{-n}{2} \log(2\pi\sigma^2) - \frac{1}{2\sigma^2} \sum_i (y_i - \hat{y}_i)^2$$



$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

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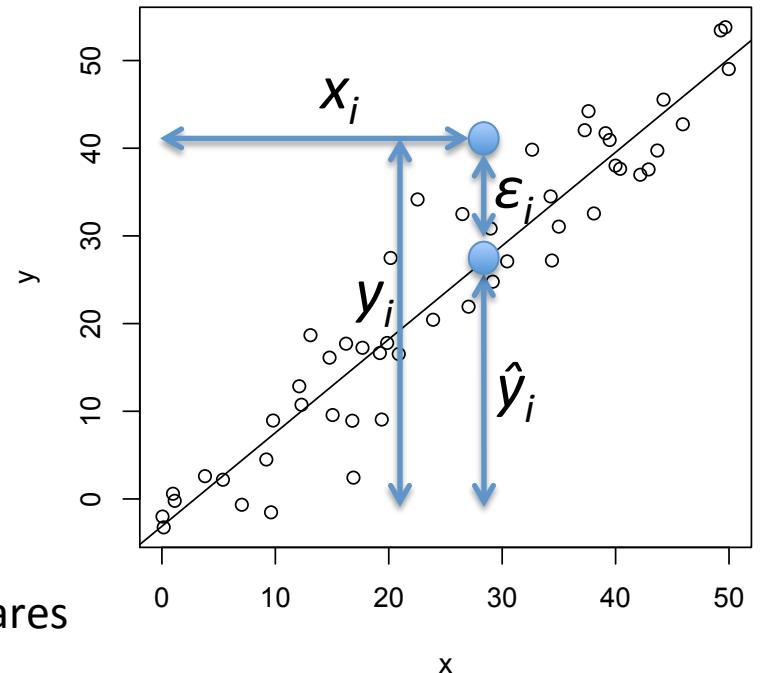
Maximise likelihood of parameters given the data

$$\mathcal{L}(\hat{\alpha}, \hat{\beta} | \mathbf{y}, \mathbf{x}) \rightarrow \max$$

$$\sum_i (y_i - \hat{y}_i)^2 \rightarrow \min$$

$$\sum_i \varepsilon_i^2 \rightarrow \min$$

Optimal parameters : minimise residual sum of squares



Maximum Likelihood and Least Squares estimates are equivalent (for Gaussian errors model)

$$y_i = \alpha + \beta x_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

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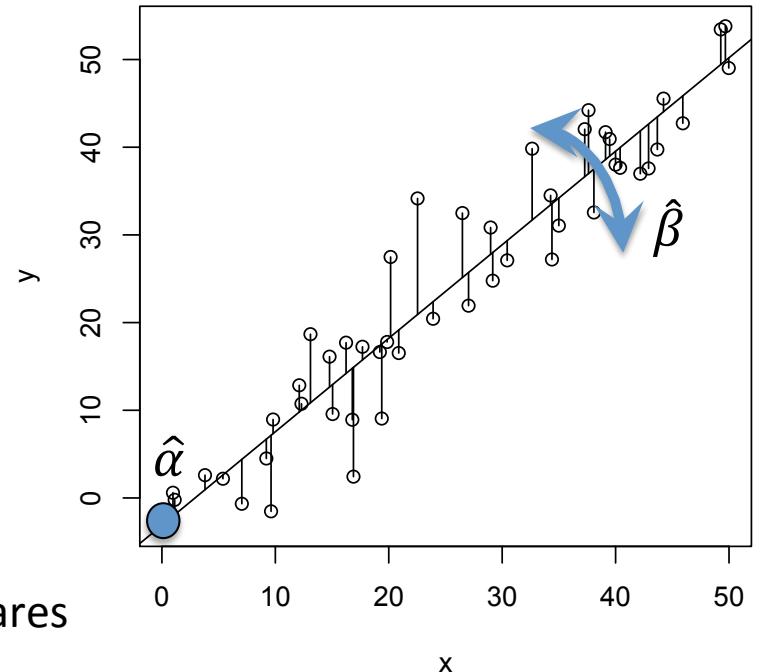
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Maximise likelihood of parameters given the data

Minimise sum of squared residuals

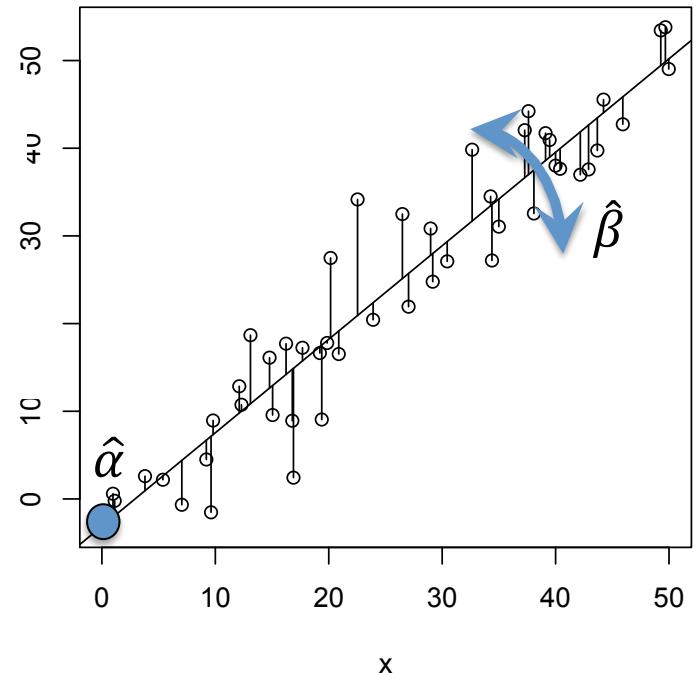
$$\sum_i \varepsilon_i^2 \rightarrow \min$$

$$\sum_i (y_i - \hat{\alpha} - \hat{\beta} x_i)^2 \underset{\hat{\alpha}, \hat{\beta}}{\rightarrow} \min$$

Final answer:

$$\hat{\beta} = \frac{\sum_i (y_i - \bar{y})(x_i - \bar{x})}{\sum_i (x_i - \bar{x})^2} = \frac{\text{cov}(\mathbf{x}, \mathbf{y})}{\text{var}(\mathbf{x})}$$

$$\hat{\alpha} = \bar{y} - \hat{\beta} \bar{x}$$



# Simple Regression:

**Example:** *Predicting timber volume of felled black cherry trees*

```
> cor(trees$Volume,trees$Girth)
[1] 0.9671194
```

```
> m1 = lm(Volume~Girth,data=trees)
> summary(m1)

Call:
lm(formula = Volume ~ Girth, data = trees)
```

Residuals:

Min	1Q	Median	3Q	Max
-8.065	-3.107	0.152	3.495	9.587

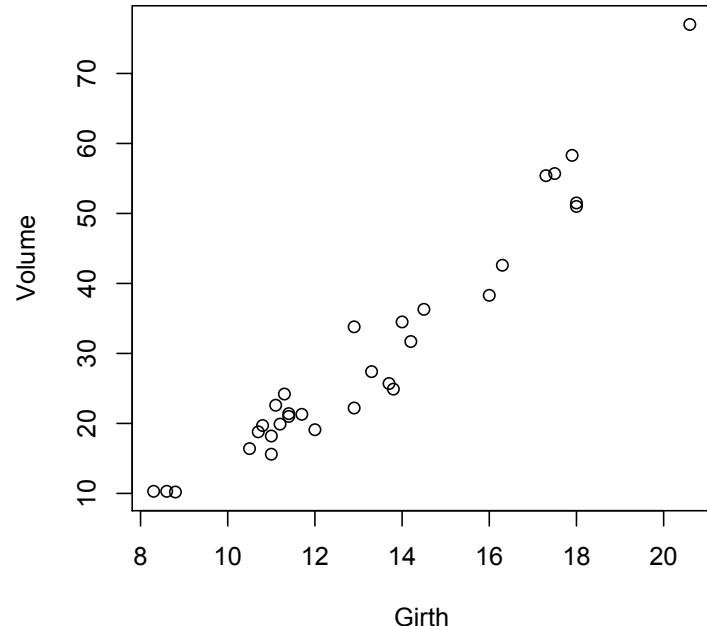
Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-36.9435	3.3651	-10.98	7.62e-12 ***
Girth	5.0659	0.2474	20.48	< 2e-16 ***

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.252 on 29 degrees of freedom  
Multiple R-squared: 0.9353, Adjusted R-squared: 0.9331  
F-statistic: 419.4 on 1 and 29 DF, p-value: < 2.2e-16



Response:  $y = \text{Volume}$   
Predictor:  $x = \text{Girth}$

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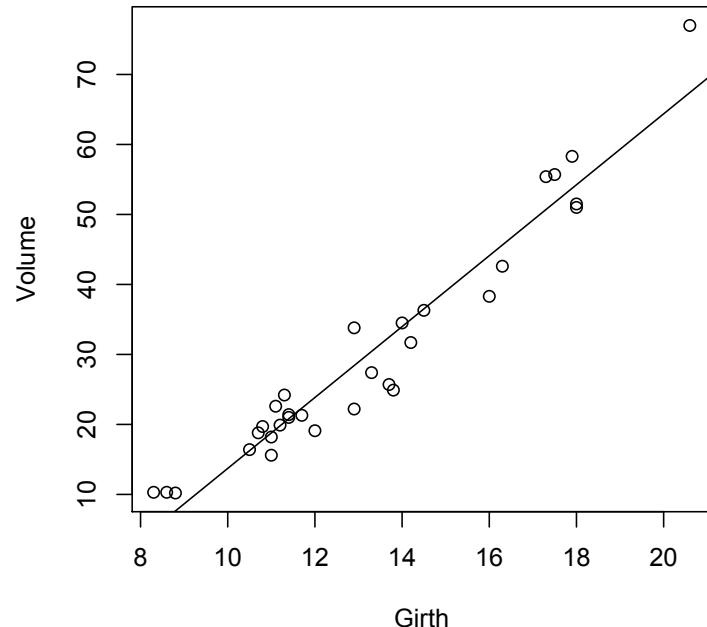
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Response:  $y = \text{Volume}$   
Predictor:  $x = \text{Girth}$

$$y = -36.9 + 5.07x$$

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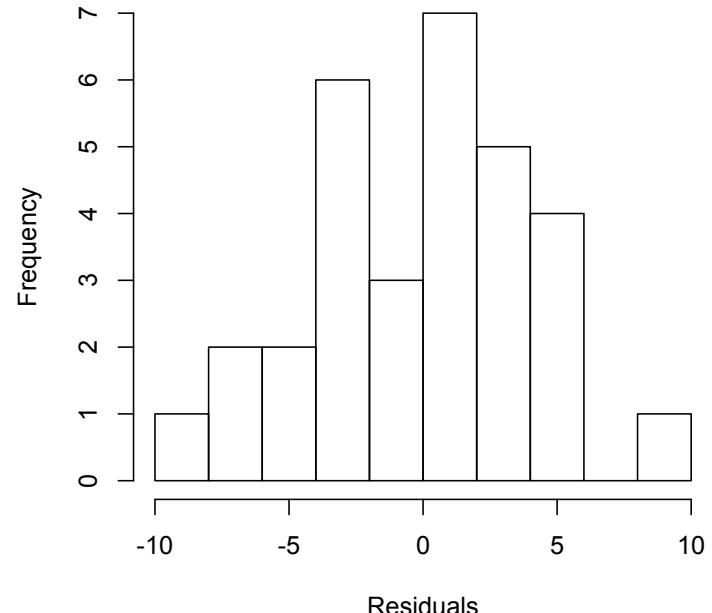
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Multiple R-squared: 0.9353, Adjusted R-squared: 0.9331  
F-statistic: 419.4 on 1 and 29 DF, p-value: < 2.2e-16

$$\sigma = 4.252$$
$$\sigma^2 = 18.1$$



Response:  $y = \text{Volume}$   
Predictor:  $x = \text{Girth}$

$$y = -36.9 + 5.07x + \varepsilon$$

$$\varepsilon \sim N(0, 18.1)$$

# Simple Regression:

**Example:** Predicting timber volume of felled black cherry trees

```
> cor(trees$Volume,trees$Girth)
[1] 0.9671194
```

```
> m1 = lm(Volume~Girth,data=trees)
> summary(m1)
```

Call:  
`lm(formula = Volume ~ Girth, data = trees)`

Residuals:

Min	1Q	Median	3Q	Max
-8.065	-3.107	0.152	3.495	9.587

Coefficients:

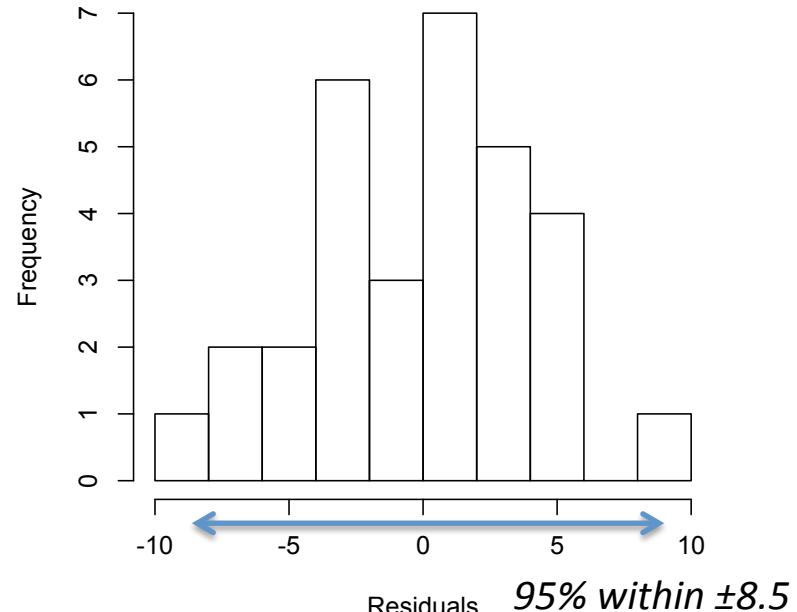
	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-36.9435	3.3651	-10.98	7.62e-12 ***
Girth	5.0659	0.2474	20.48	< 2e-16 ***

---

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# Linear Regression:

## Assumptions:

1. Model is linear in parameters.

$$y = \alpha + \beta x + \varepsilon$$

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# Linear Regression:

## Assumptions:

1. Model is linear in parameters.
2. Gaussian error model.

$$y = \alpha + \beta x + \varepsilon$$

$$\varepsilon \sim N(\mathbf{0}, \sigma^2 \mathbf{I})$$

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## Assumptions:

1. Model is linear in parameters.
2. Gaussian error model.
3. Additive error model.

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$$\varepsilon \sim N(\mathbf{0}, \sigma^2 \mathbf{I})$$

$$y = \alpha + \beta x\varepsilon$$

$$y = \alpha + \beta x^\varepsilon$$

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$$\text{Cov}(\varepsilon_i, \varepsilon_j) = 0$$

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5. Homoscedasticity.

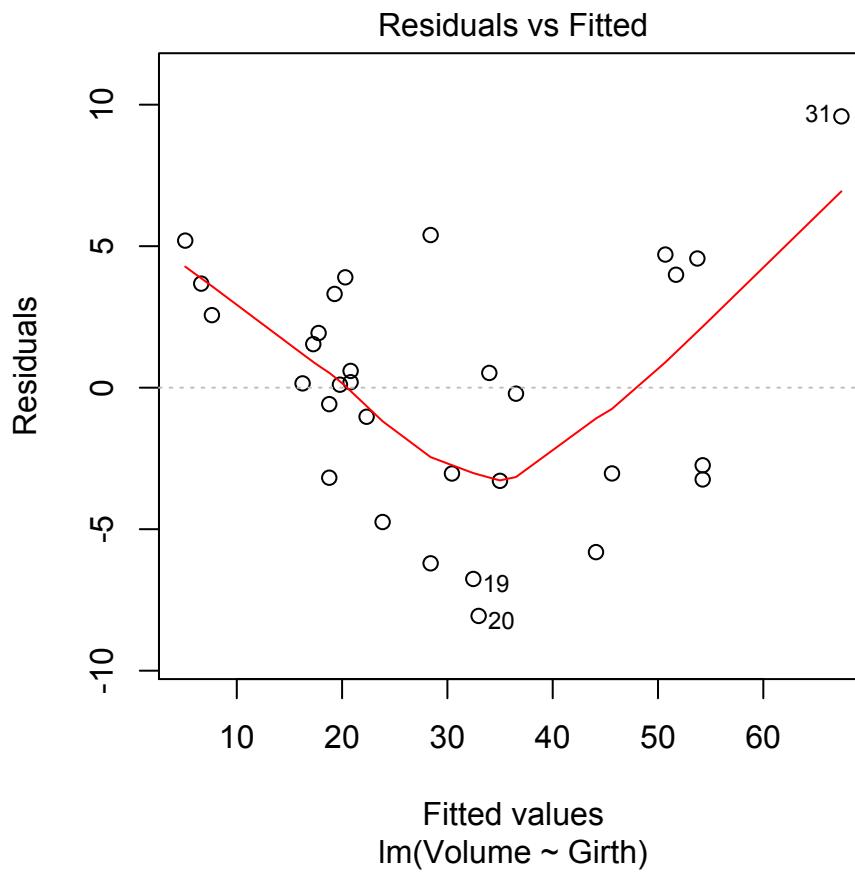
$$\text{Var}(\varepsilon|x) = \sigma^2 \mathbf{I}$$

Homogeneity / stability of variance of the residuals

# Testing Assumptions: diagnostic plots

## 1. Residuals vs Fitted Values

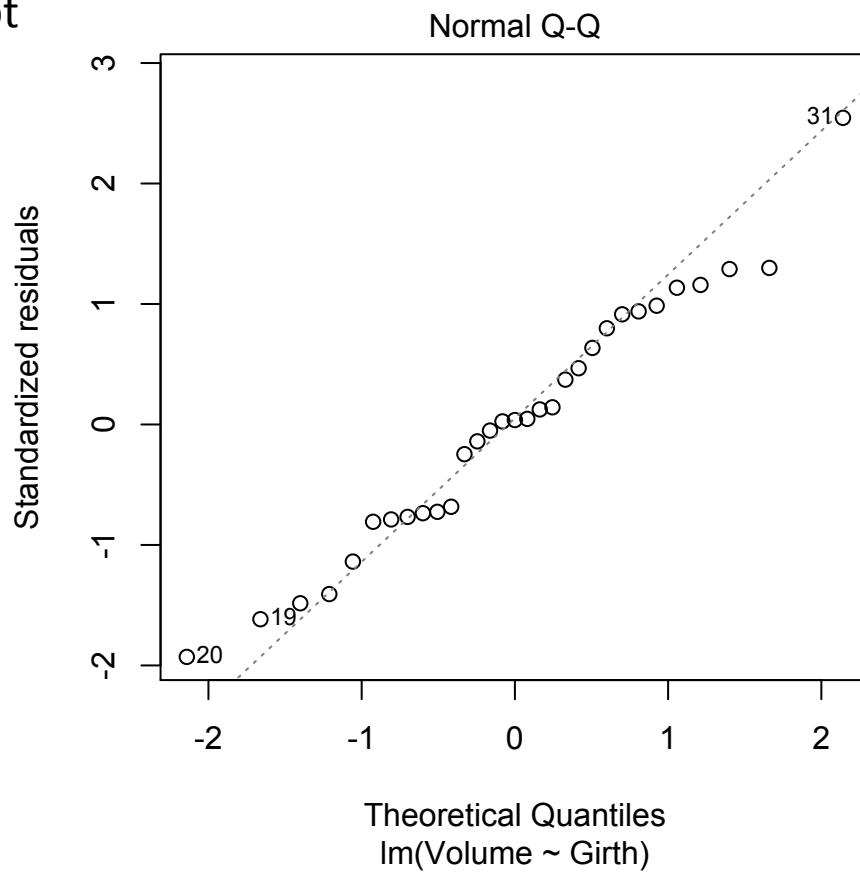
- Should not be related
- No visible pattern
- Mean residual = zero
- Constant variance



# Testing Assumptions: diagnostic plots

1. Residuals vs Fitted Values
2. Normal Quantile-Quantile plot

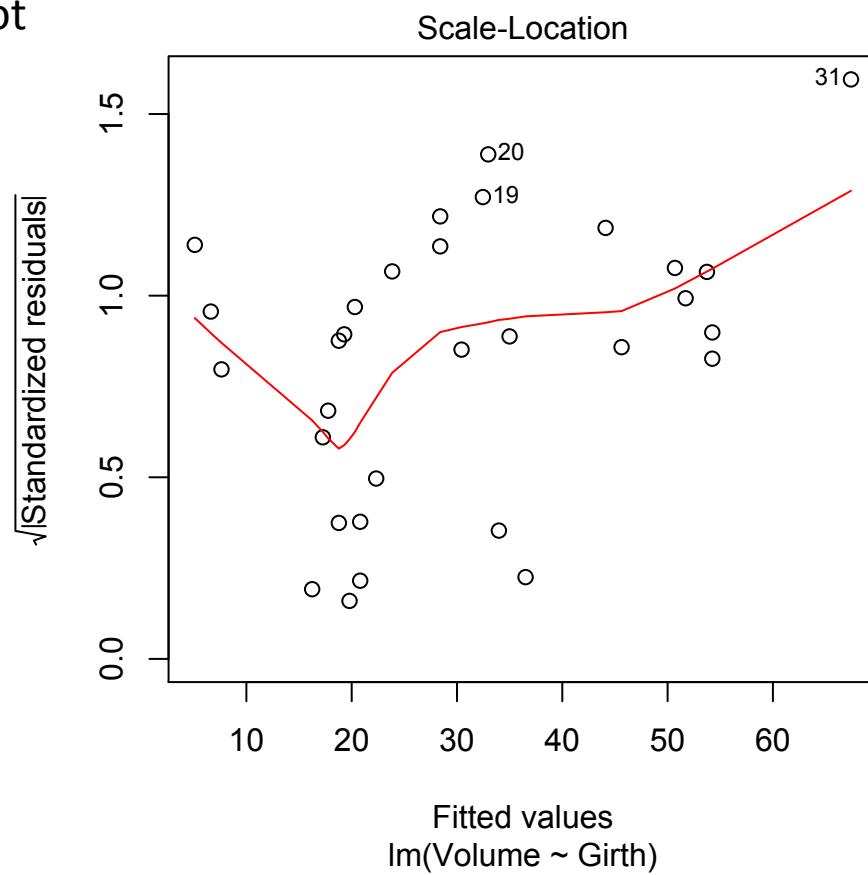
- Visual test for Normality
- No strong trends/departures



# Testing Assumptions: diagnostic plots

1. Residuals vs Fitted Values
2. Normal Quantile-Quantile Plot
3. Scale-Location Plot

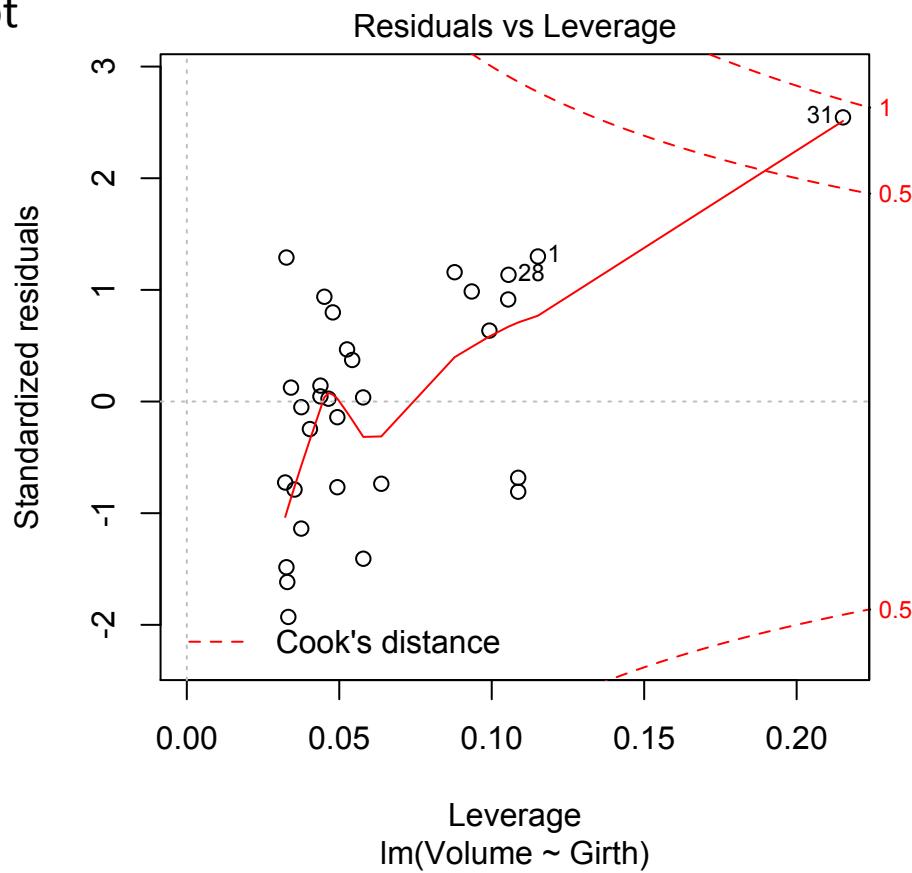
- Test for homoscedasticity
- Should be constant,  $\approx 1$
- No trend



# Testing Assumptions: diagnostic plots

1. Residuals vs Fitted Values
2. Normal Quantile-Quantile Plot
3. Scale-Location Plot
4. Index Plot of Cook's Distance

- Measures the influence of a particular observation
- Extreme x-vals : high leverage
- May inform outlier rejection



# Modelling Non-Linear Relationships

Linear models can be used to describe non-linear relationships...

$$y = \alpha + \beta x + \varepsilon$$

$$y = \alpha + \beta x^2 + \varepsilon$$

$$y = \alpha + \beta \log(x) + \varepsilon$$

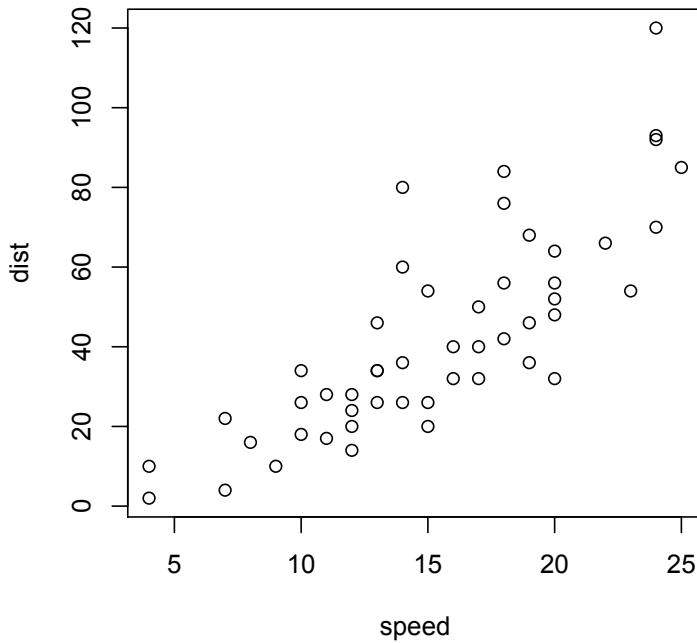
$$\log(y) = \alpha + \beta \sqrt{x} + \varepsilon$$

Applying transformations to response and/or predictor variables can be useful to:

- Linearise the data, i.e. make the relationship between variables more linear.
- Stabilise the variance of the residuals, so that  $\sigma^2$  doesn't depend on the independent variable.
- Normalise the distribution of the residuals

# Modelling Non-Linear Relationships

**Example:** *Stopping distance of cars versus speed (mph)*

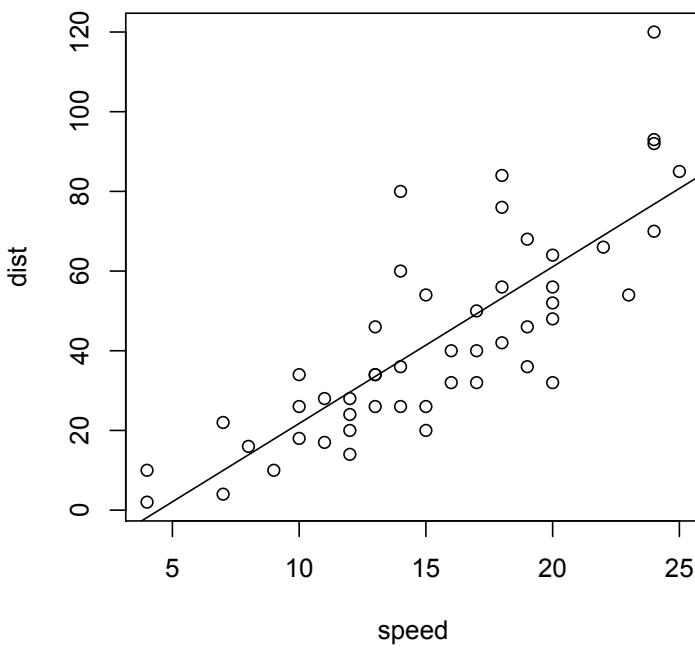


Response:  $y = \text{distance}$

Predictor:  $x = \text{speed}$

# Modelling Non-Linear Relationships

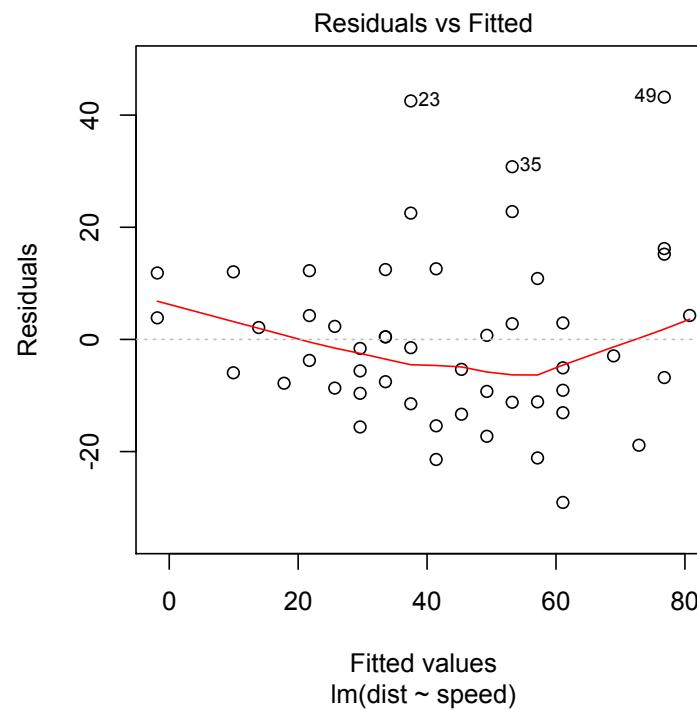
Example: Stopping distance of cars versus speed (mph)



Response:  $y = \text{distance}$   
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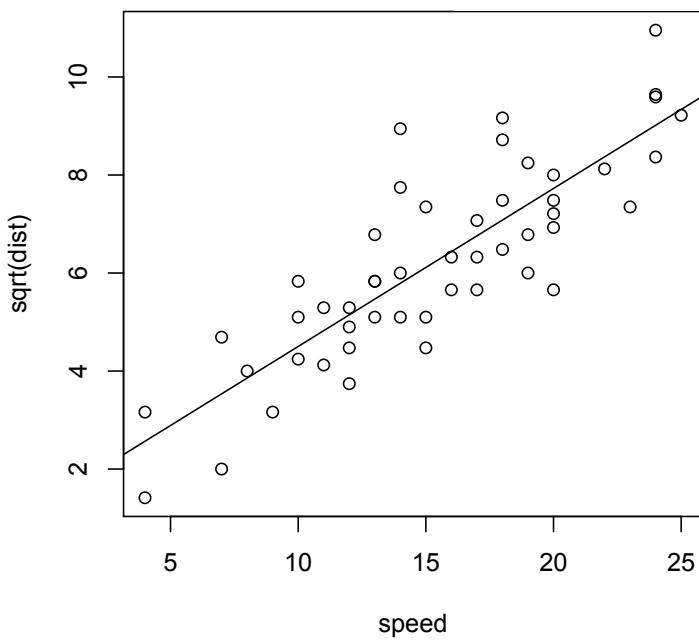
$$y = \alpha + \beta x + \varepsilon$$

$$R^2 = 0.651$$



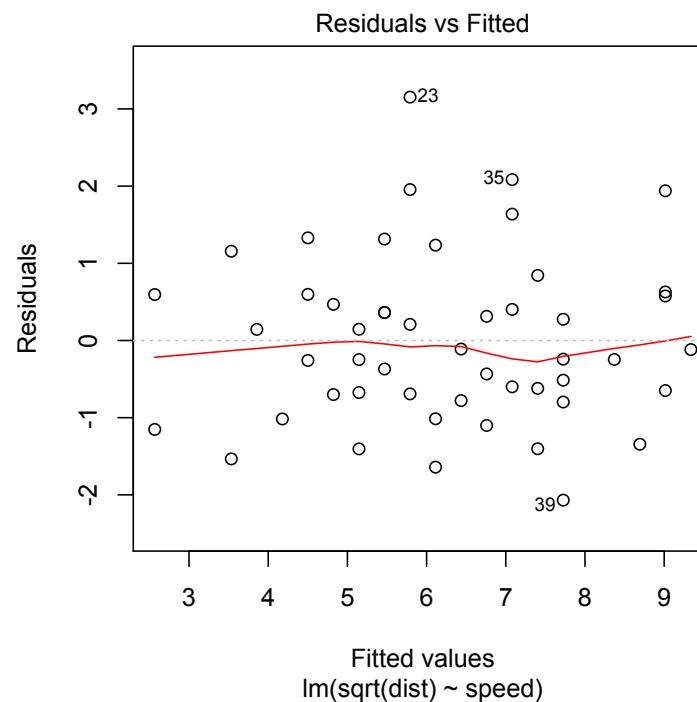
# Modelling Non-Linear Relationships

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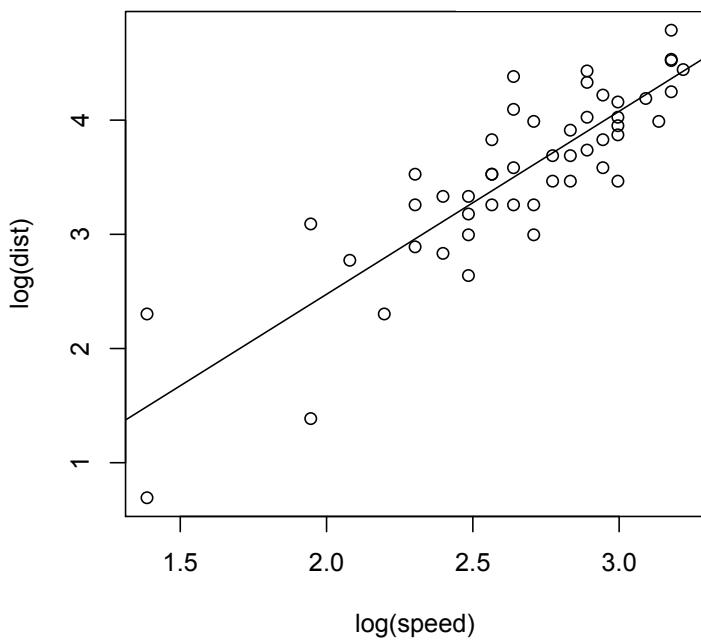
Response:  $y = \text{distance}$   
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$$y = \alpha + \beta x + \varepsilon \quad R^2 = 0.651$$
$$\sqrt{y} = \alpha + \beta x + \varepsilon \quad R^2 = 0.709$$



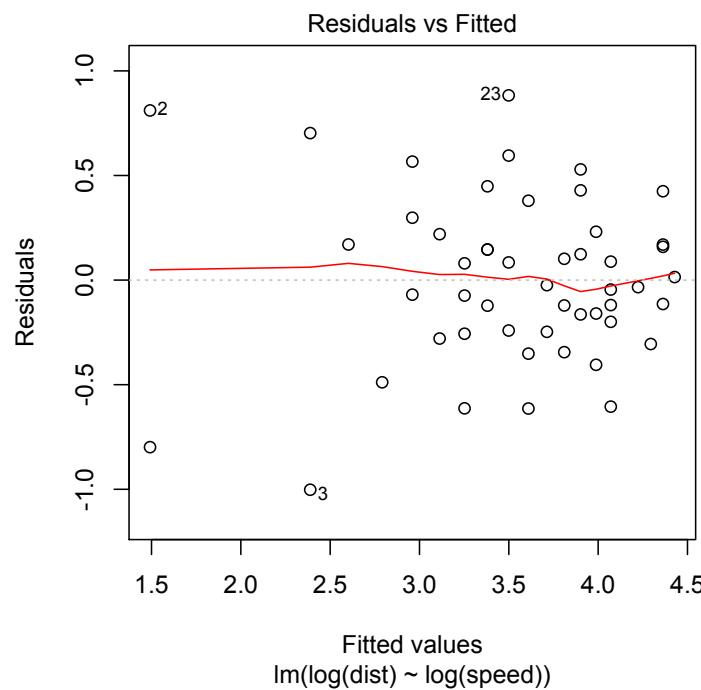
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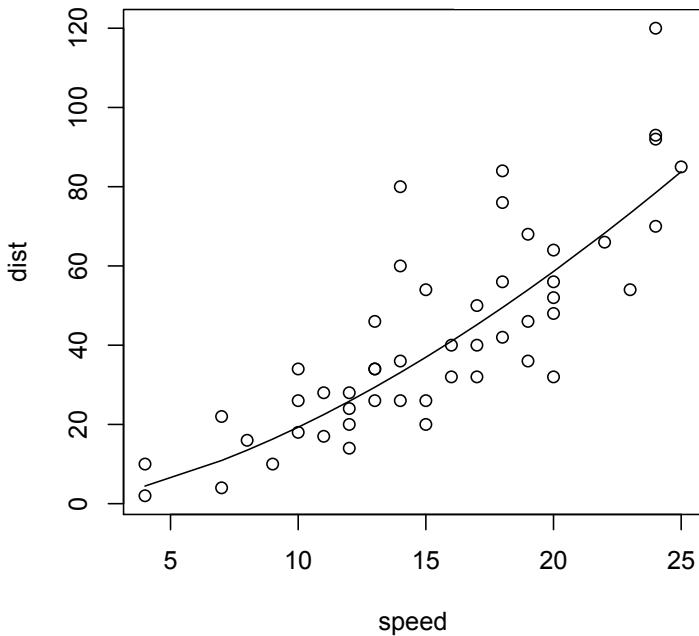
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Call:  
`lm(formula = log(dist) ~ log(speed), data = cars)`

Residuals:

Min	1Q	Median	3Q	Max
-1.00215	-0.24578	-0.02898	0.20717	0.88289

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.7297	0.3758	-1.941	0.0581 .
log(speed)	1.6024	0.1395	11.484	2.26e-15 ***

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1  
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Residual standard error: 0.4053 on 48 degrees of freedom  
Multiple R-squared: 0.7331, Adjusted R-squared: 0.7276  
F-statistic: 131.9 on 1 and 48 DF, p-value: 2.259e-15

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Can you use simple regression to fit this model?

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Multiplicative error model

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Multiplicative error model

$$\log(y) = \log(\alpha) + \beta \log(x) + \log(\varepsilon)$$

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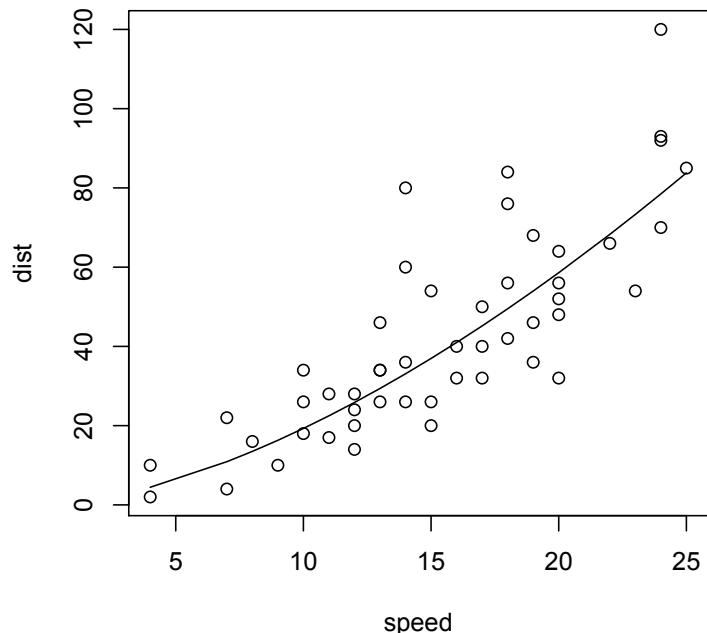
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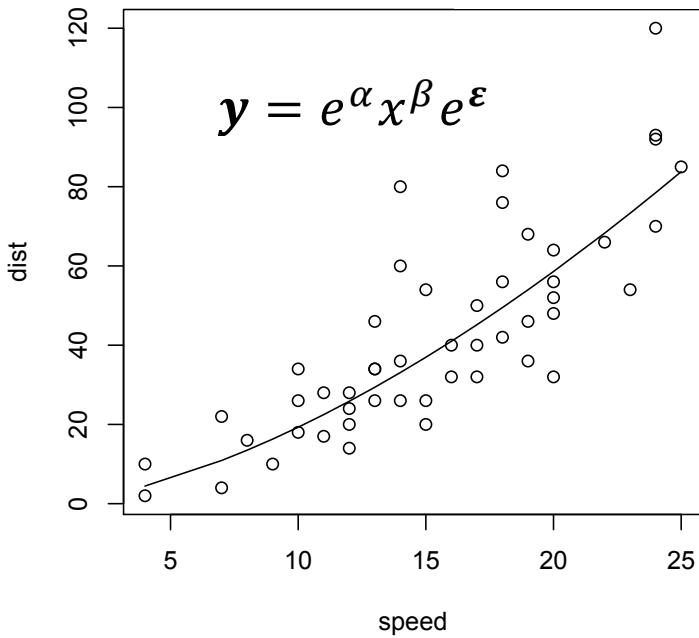
Yes, so long as  $\log(\varepsilon) \sim N(0, \sigma^2)$

Error model is log-Normal.



# Modelling Non-Linear Relationships

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# Simple Regression in R:

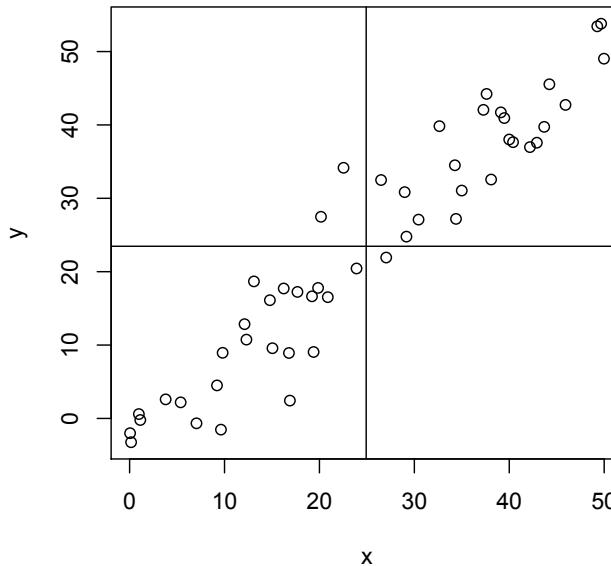
## Correlation Coefficients:

### R functions:

**plot(x,y)**

**cor(x,y)**

**cor.test(x,y)**



data: x and y

t = 17.613, df = 48, p-value < 2.2e-16

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.8802556 0.9602168

sample estimates:

cor

0.9305923

# Simple Regression in R:

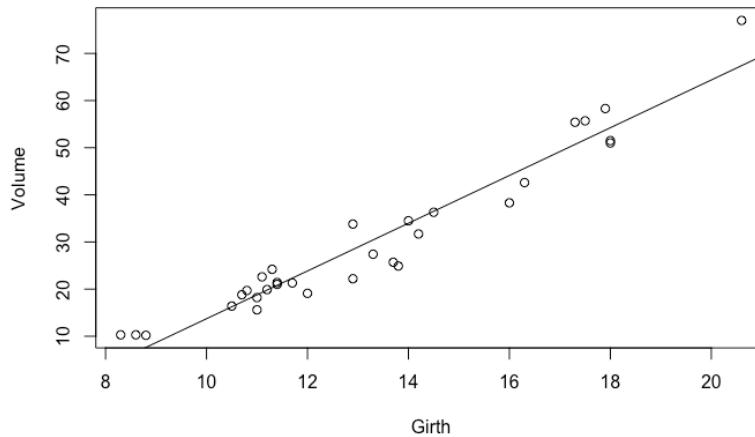
R functions:

**plot(x,y)**

**m1 = lm(y~x)**

**abline(m1)**

**summary(m1)**



Call:

`lm(formula = Volume ~ Girth, data = trees)`

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-8.065	-3.107	0.152	3.495	9.587

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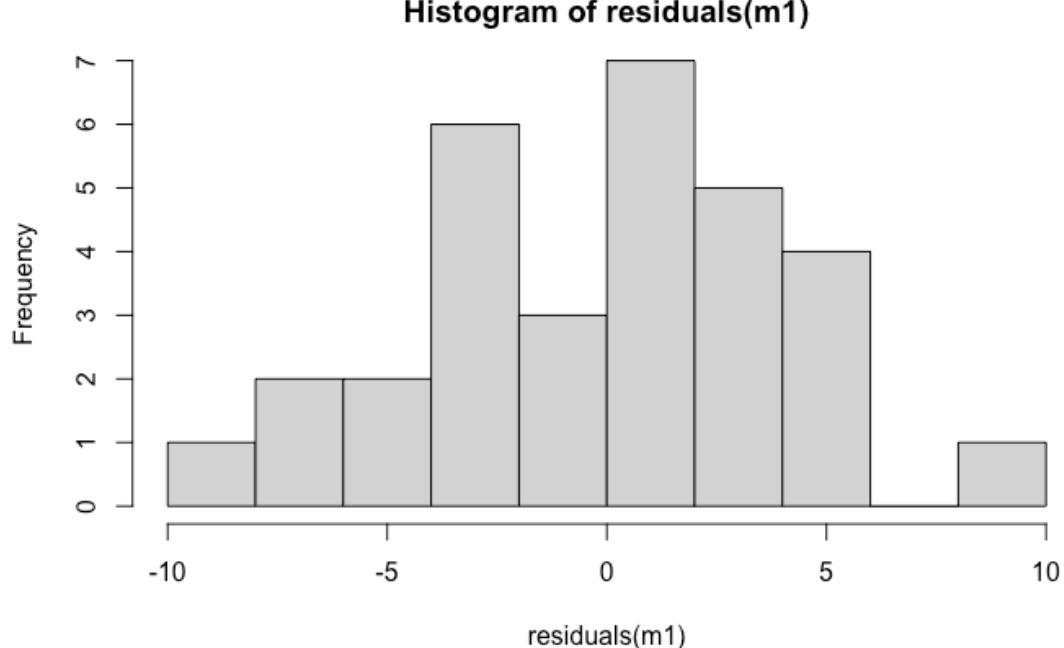
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**hist(r1)**

**plot(m1)**

