Linear modeling I

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BIO5312, FALL 2017

General notes on Homework

Do not use Rstudio-specific commands in R chunks

- View() is not an R command
- May prevent from knitting

Only set working directory once

- Add to top chunk with "root.dir"

Load all libraries in the top chunk

Chi-squared assumptions refer to **expected** counts

Don't give yourself more work than you have to

Don't make your hypotheses fancy

When to use median vs. mean for directional conclusions?

Announcing Final Project (25%)

Identify a dataset and ask **four scientific questions** about the data

- Use any of the statistical approaches we have learned to answer each question
- Make a descriptive figure for each scientific question

Scientific questions

Recall HW7:

- Do PHA levels tend to differ between the birds that received supplemental carotenoids and those that did not? Based on your results, can you infer anything about immune differences between birds that did and did not receive carotenoids?
  - **Use a Mann Whitney U test to answer the scientific question**
  - "Run a Mann Whitney U test on PHA levels between bird treatments" is not a scientific question

- What figure might be good to make here?
- What figures would not be good here?

Final project proposal

Homework due 11/28 will be a proposal

- Identify your dataset and give 1-2 paragraphs of background
  - **IN YOUR OWN WORDS**
- Pose four scientific questions
- Explain how you will solve each question
  - What statistical procedure and why
- Indicate how you will visualize your data

3-4 sentences total per question

Around 1 written page, single-spaced.

Updated schedule

Date	Topic
10/24	Linear modeling I
10/31	Linear modeling II and logistic regression
11/7	Model selection and evaluation
11/14	Principal Components Analysis (PCA) and clustering
11/21	Thanksgiving break
11/28	Advanced R grabbag and/or overflow
12/5	Advanced R grabbag and/or in-class office hours for final project **Email me for special topic requests.**
12/12	Final project due (by 11:59 pm on 12/12)

Linear Modeling

ANOVA and friends

Correlation

Regression

Multiple regression

ANOVA: Analysis of Variance

Used to compare more than 2 means (among *k* groups)

Ho: All means are the same, i.e. $\mu_1 = \mu_2 = \ldots = \mu_k$

Ha: At least one mean is different, i.e. at least one $\mu_{<1\text{-}k>}$ differs

Why "can't" we use a *t*-test?

- We can do all the comparisons and use a P-value correction
- ANOVA is preferred

ANOVA Example

A clinical trial asks if there is a difference in mean daily calcium intake in adults with normal bone density, adults with osteopenia, and adults with osteoporosis. Each participant's daily calcium intake is measured based on reported food intake.

Normal Bone Density	Osteopenia	Osteoporosis
1200	1000	490
1000	1100	650
980	700	200
900	800	300
750	500	400
800	700	350

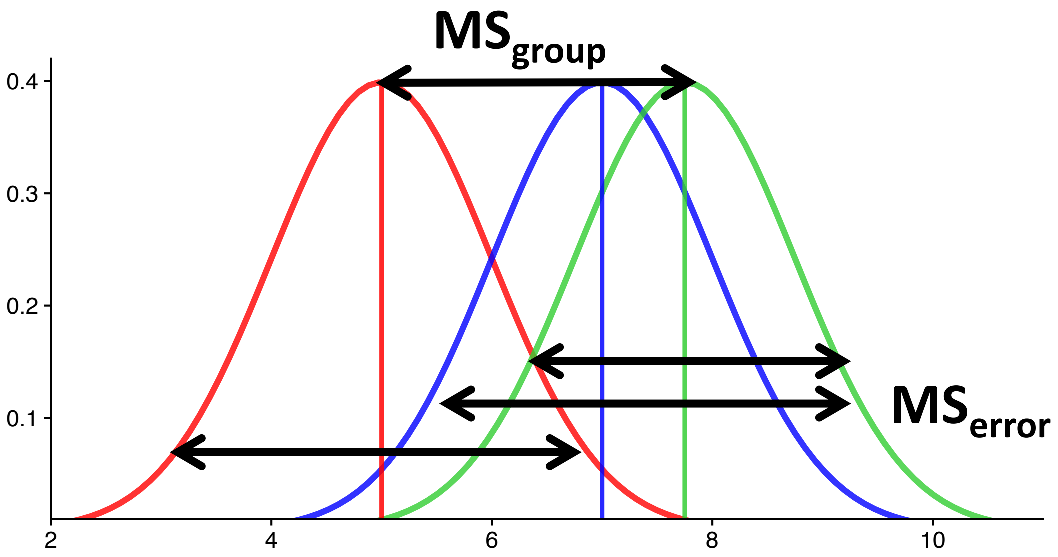
Is there a difference in mean calcium intake across groups?

ANOVA compares sources of *variance* using the **F statistic**

Variance *among* groups is the **group mean square** (MS_{group})

Variance *within* each group is the **error mean square** (MS_{error})

- Pooled sample variance



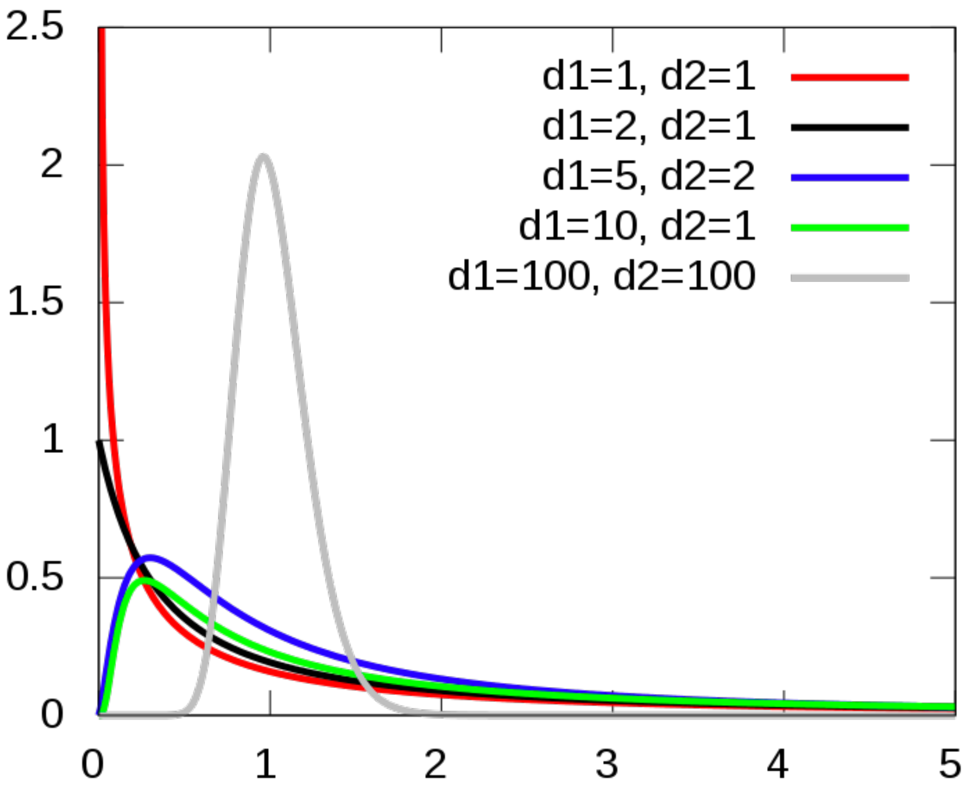
$$F = \frac{MS_{group}}{MS_{error}}$$

The F statistic

$$\boldsymbol{F} = \frac{\boldsymbol{MS_{group}}}{\boldsymbol{MS_{error}}}$$

df group = $k - 1$

df error = $N - k$ = $\sum n - 1$



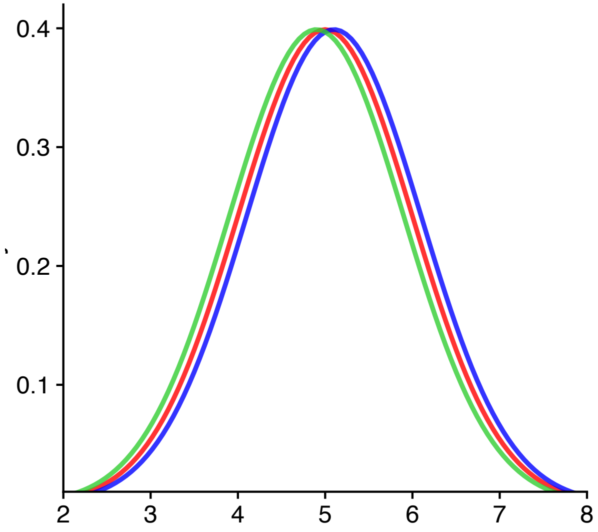
We use only the upper tail for P-value

https://en.wikipedia.org/wiki/F-distribution#/media/File:F-distribution_pdf.svg

Interpreting the **F** statistic

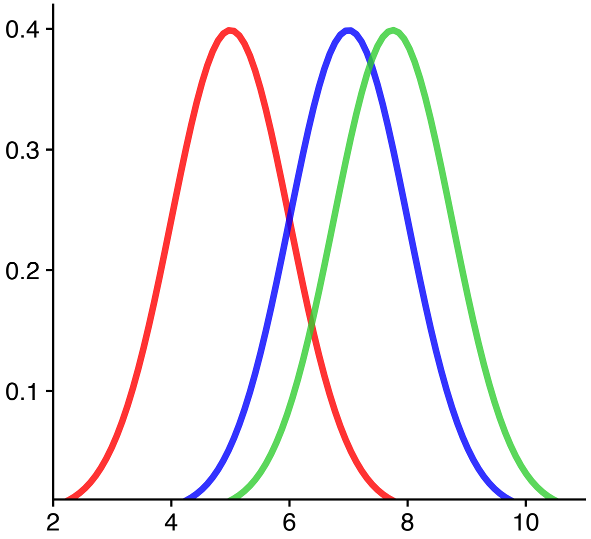
If H_O is true, $F \cong 1$

$MS_{group} \cong MS_{error}$



If H_O is false, $F > 1$

$MS_{group} > MS_{error}$



Computing the **F** statistic

$$F = \frac{MS_{group}}{MS_{error}} \qquad MS = \frac{SS}{df}$$

Sum of squares

$$MS_{error} = \frac{\boxed{\sum s_i^2 (n_i - 1)}}{N - k}$$

$$MS_{group} = \frac{\boxed{\sum n_i (\bar{X}_i - \bar{X})^2}}{k - 1}$$

s_i^2 =Standard deviation of group i

$df_i = n_i - 1$, where n_i = sample size of group i

$\bar{X}_i$ = mean of group i

$\bar{X}$ = grand mean (mean of *all* numbers)

Error degrees of freedom =

$$df_{error} = \sum df_i = \sum (n_i - 1) = N - k$$

Computing the F statistic

$$F = \frac{MS_{group}}{MS_{error}} \qquad MS = \frac{SS}{df}$$

$$MS_{error} = \frac{\boxed{\sum s_i^2 (n_i - 1)}}{N - k}$$

$$MS_{group} = \frac{\boxed{\sum n_i (\bar{X}_i - \bar{X})^2}}{k - 1}$$

Normal Bone Density	Osteopenia	Osteoporosis
1200	1000	490
1000	1100	650
980	700	200
900	800	300
750	500	400
800	700	350

Running the ANOVA

Ho: Groups have the same mean calcium intake.

Ha: At least one group has a different calcium intake.

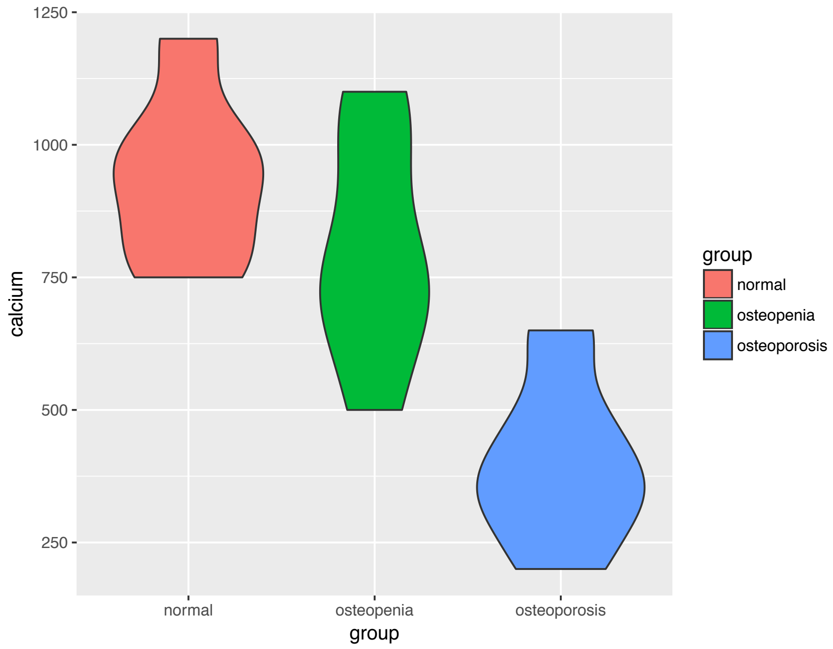
```
data <- tibble("normal"       = c(1200, 1000, 980, 900, 750, 800),
               "osteopenia"   = c(1000, 1100, 700, 800, 500, 700),
               "osteoporosis" = c(490, 650, 200, 300, 400, 350))

data %>% gather(group, calcium, normal:osteoporosis) -> tidy.data
```

```
        group calcium
1      normal    1200
2      normal    1000
3      normal     980
4      normal     900
5      normal     750
6      normal     800
7  osteopenia    1000
8  osteopenia    1100
9  osteopenia     700
10 osteopenia     800
11 osteopenia     500
12 osteopenia     700
...
```

Visualize the data

It is **always** the right idea to view your data before modeling it

```
ggplot(tidy.data, aes(x = group, y = calcium, fill= group)) + geom_violin()
```



Running the ANOVA

```
> aov(calcium ~ group, data = tidy.data)
Call:
   aov(formula = calcium ~ group, data = tidy.data)


Terms:
                            error
                   group Residuals
Sum of Squares  944144.4  493166.7
Deg. of Freedom        2        15


Residual standard error: 181.3223
Estimated effects may be unbalanced
```

Obtaining the ANOVA table

```
> summary( aov(calcium ~ group, data = tidy.data) )
```

```
> 1-pf(14.36, 2, 15)
[1] 0.0003277806
```

```
                Df Sum Sq Mean Sq F value   Pr(>F)
      group      2 944144  472072   14.36 0.000328 ***
error Residuals 15 493167   32878
      ---
      Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

$$MS_{group} = \boxed{\frac{\sum n_i\,(\bar{X}_i - \bar{X})^2}{k-1}}$$

$$MS_{error} = \boxed{\frac{\sum s_i^2\, df_i}{N-k}}$$

$$F = \frac{MS_{group}}{MS_{error}}$$

Reports and conclusions

Our P = 0.000328, which is less than alpha. We reject the null hypothesis and we have evidence that at least one mean (normal bone density, osteopenia, or osteoporosis calcium intake) differs from the other.

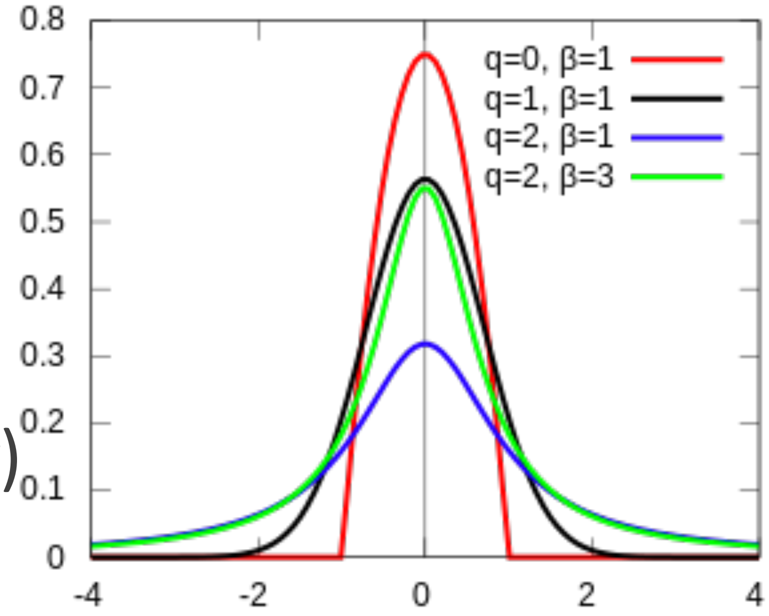
Unplanned comparisons with the Tukey-Kramer Method

AKA Tukey's test, Tukey's method, Tukey's HSD (honest significant difference) test

Tests all pairs of means

- Normal vs. osteopenia
- Normal vs. osteoporosis
- Osteopenia vs. osteoporosis

Roughly, multiple t-tests where FWER is controlled but using the *q*-statistic (similar to *t*)



Running Tukey's test on ANOVA

```
> TukeyHSD( aov(calcium ~ group, data = tidy.data) )
  Tukey multiple comparisons of means
    95% family-wise confidence level


Fit: aov(formula = calcium ~ group, data = tidy.data)

$group
                             diff       lwr       upr     p adj
osteopenia-normal       -138.3333 -410.2534  133.5867 0.4054988
osteoporosis-normal     -540.0000 -811.9200 -268.0800 0.0003238
osteoporosis-osteopenia -401.6667 -673.5867 -129.7466 0.0043335
```

Reports and conclusions, round 2

Our P = 0.000328, which is less than alpha. We reject the null hypothesis and we have evidence that at least one mean (normal bone density, osteopenia, or osteoporosis calcium intake) differs from the other.

After running the *post-hoc* Tukey's test, we find that osteoporosis groups have a significantly higher average calcium intake than normal groups (P=0.0003), and that osteoporosis groups have a significantly higher average calcium intake than osteopenia groups P=0.004). However, we do not find a significant difference in calcium intake between normal and osteopenia groups.

ANOVA assumptions

Samples are random

Samples are normally distributed

- Robust to violations when study is **large**

Samples have the same variance

- Robust to violations when study is **balanced**

Kruskal-Wallis is the non-parametric alternative

```
> kruskal.test(calcium ~ as.factor(group), data = tidy.data)


Kruskal-Wallis rank sum test


data:  calcium by as.factor(group)
Kruskal-Wallis chi-squared = 11.439, df = 2, p-value = 0.003281
```

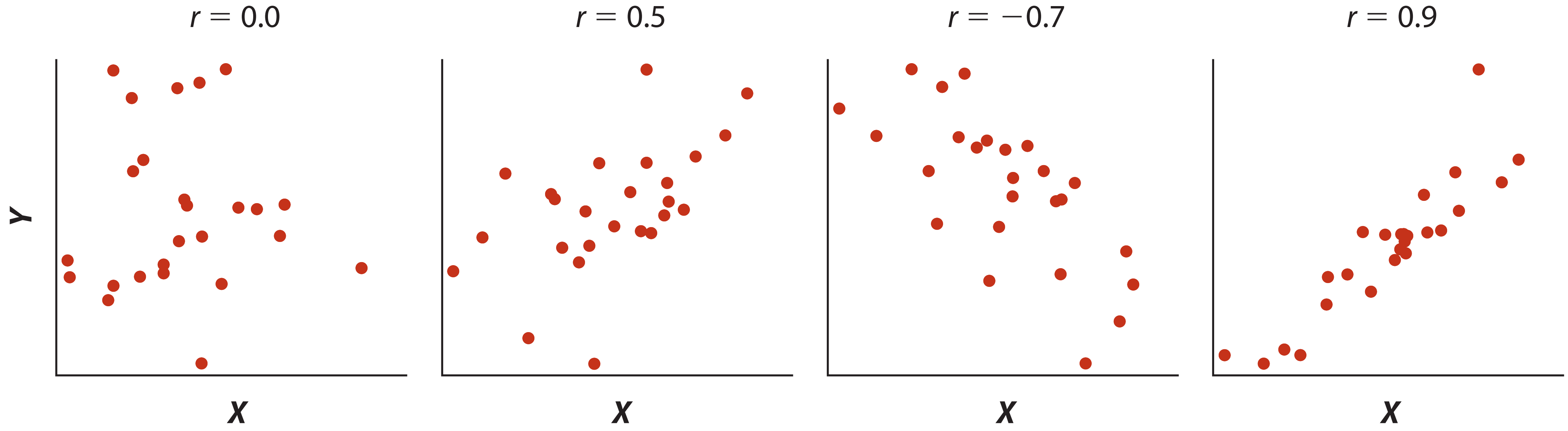
Unless something is **really really weird**, you "can" use ANOVA

Exercise break

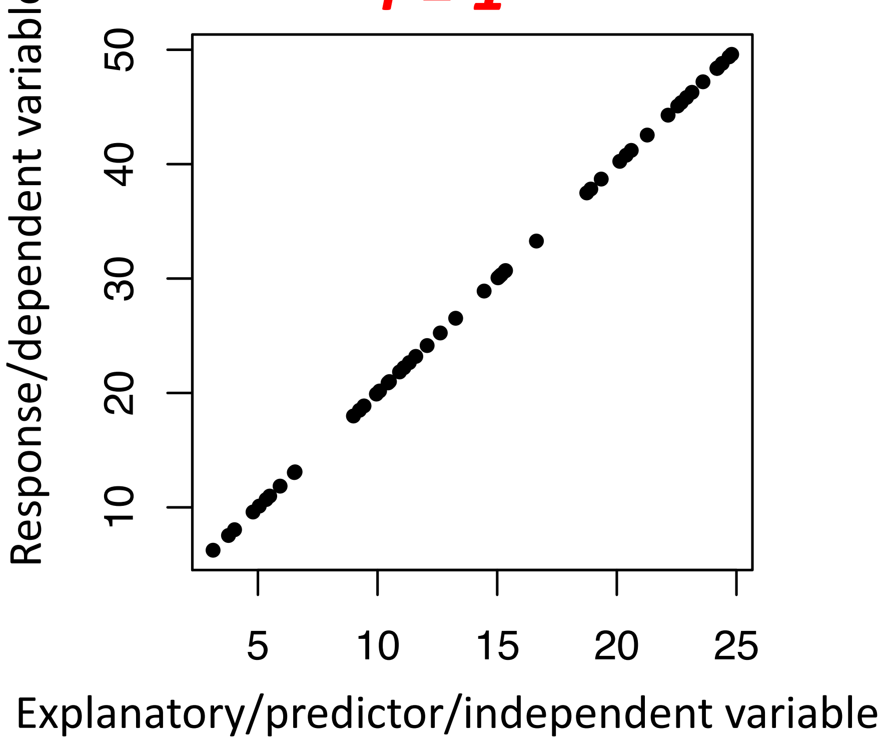
Correlation

Measures the strength and direction of the **linear association** between two numeric variables

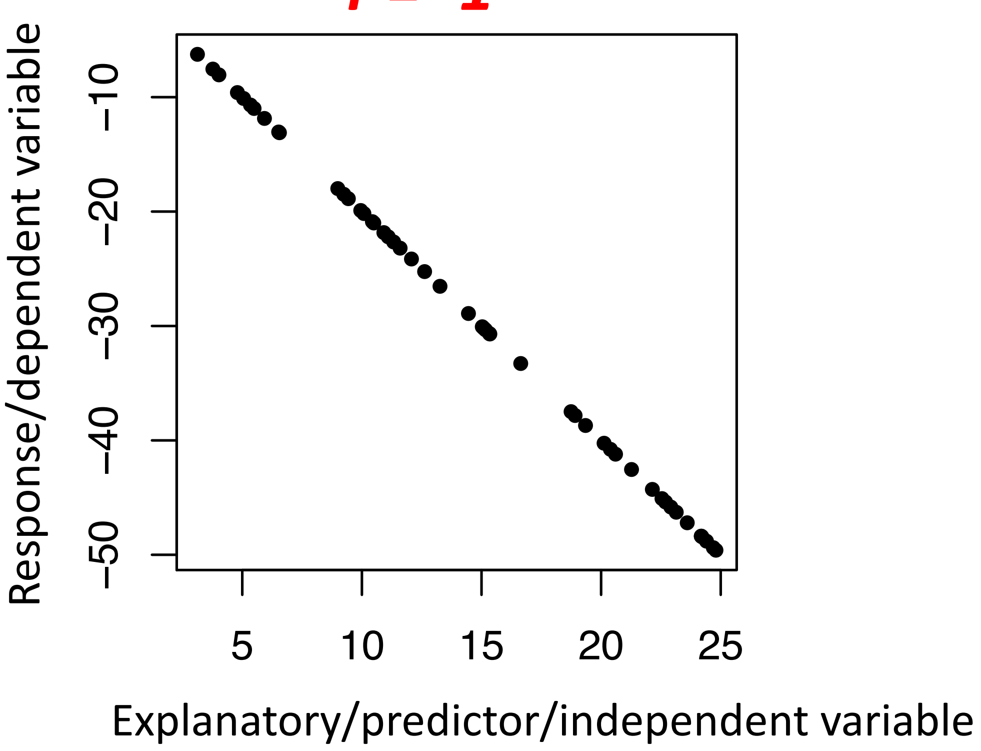
$$-1 \le r \le 1$$



Perfect correlations

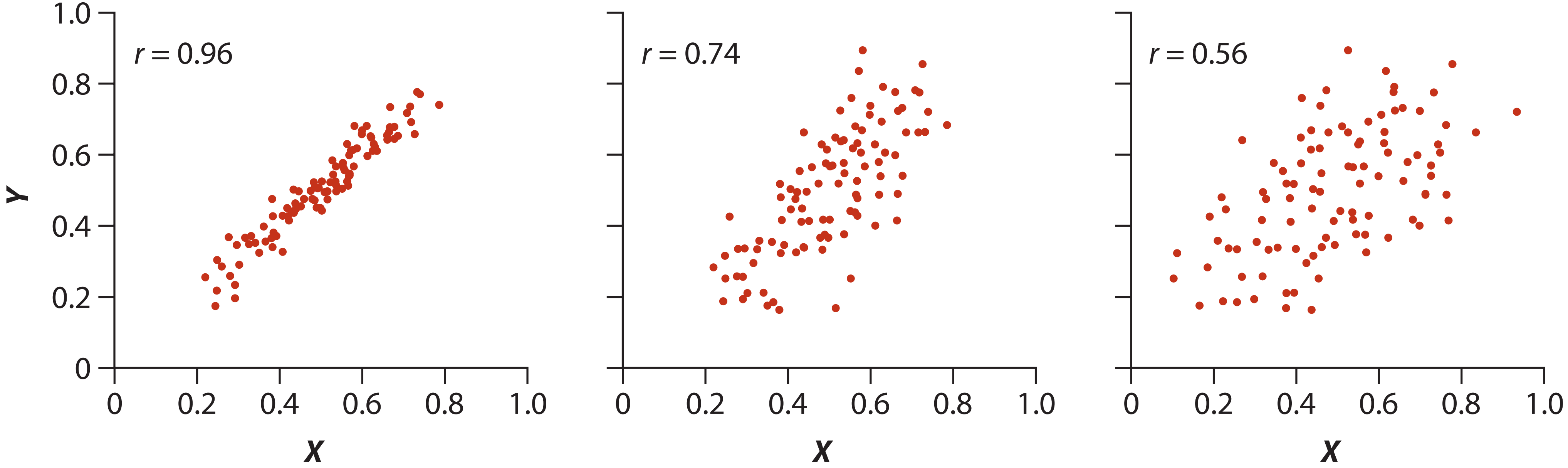


r = 1



r = -1

Variability (error) has a substantial influence



Calculating the correlation coefficient

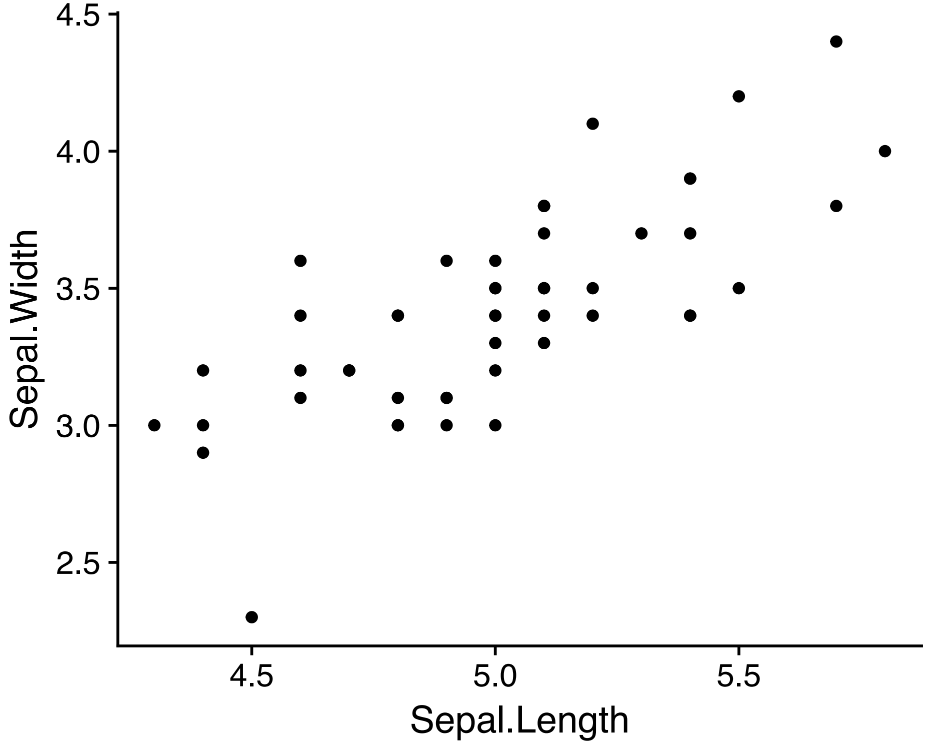
$$\boldsymbol{r = \frac{cov(X,Y)}{s_X s_Y}}$$

$$\boldsymbol{= \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}\sqrt{\frac{\sum(Y_i - \bar{Y})^2}{n-1}}}}$$

$$\boldsymbol{= \frac{1}{n-1}\frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2}\sqrt{\sum(Y_i - \bar{Y})^2}}}$$

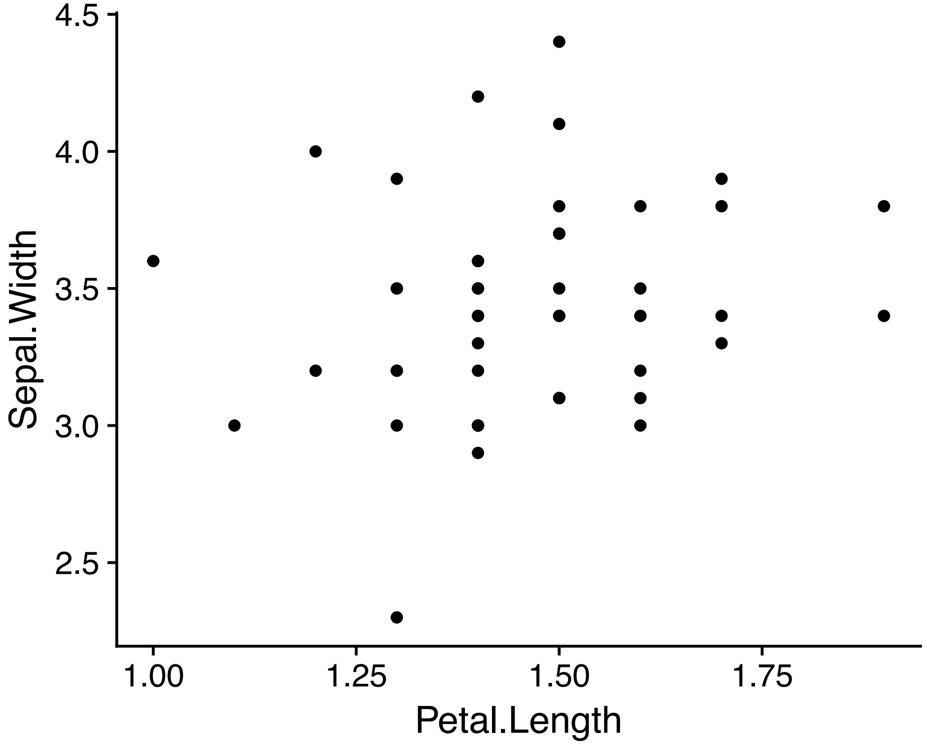
Example: correlation between irises

```
> setosa <- iris %>% filter(Species == "setosa")
> cor(setosa$Sepal.Length, setosa$Sepal.Width)
   [1] 0.7425467


> cor(setosa$Sepal.Width, setosa$Sepal.Length)
[1] 0.7425467
```



Example: correlation between irises

```
> cor(setosa$Petal.Length, setosa$Sepal.Width)
   [1] 0.1777
```



Hypothesis testing with correlations

H_O: Petal length and sepal width are not correlated (r=0)
H_A: Petal length and sepal width are correlated (r!=0)

```
> cor.test(setosa$Sepal.Width, setosa$Sepal.Length)
Pearson's product-moment correlation

data:  setosa$Sepal.Width and setosa$Sepal.Length
t = 7.6807, df = 48, p-value = 6.71e-10
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.5851391 0.8460314
sample estimates:
      cor
0.7425467
```

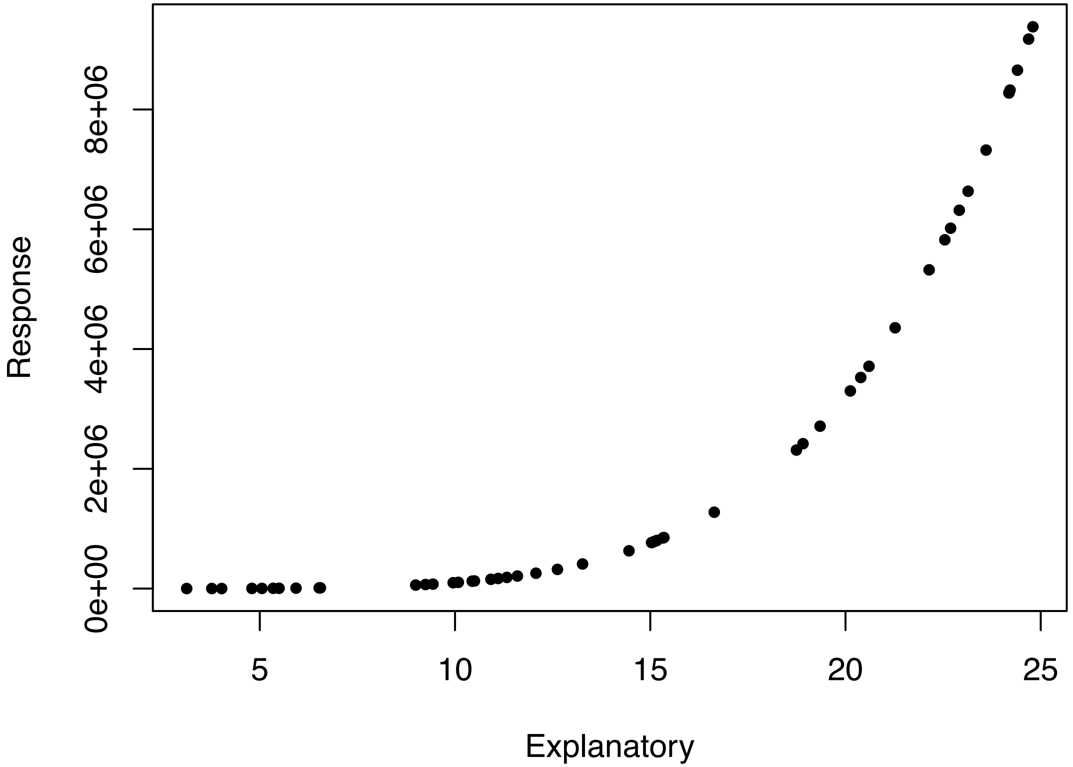
With P=6.7e-10, which is much less than 0.05, we reject the null hypothesis. We find evidence that setosa sepal widths and sepal lengths are corrected. The correlation coefficient r=0.74, with a 95% CI of [0.58, 0.85] This value is above 0, indicating a positive relationship, and it is fairly large, indicating a strong correlation.

Hypothesis testing

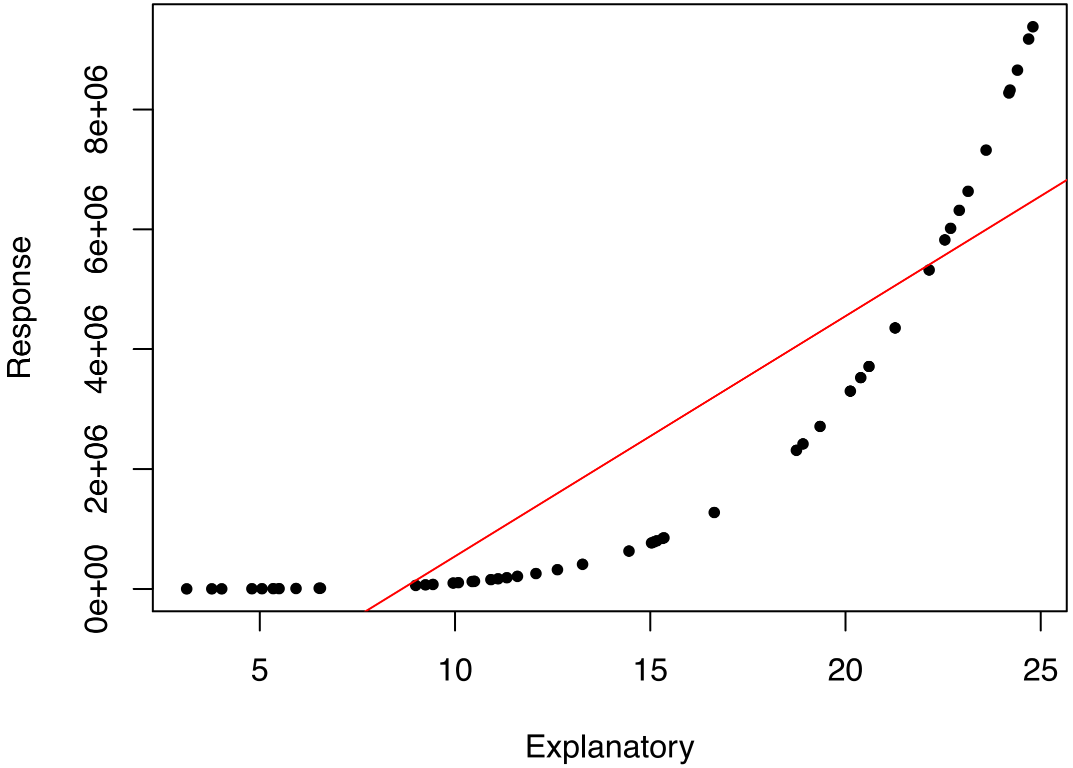
```
> cor.test(setosa$Petal.Length, setosa$Sepal.Width)

Pearson's product-moment correlation

data:  setosa$Petal.Length and setosa$Sepal.Width
t = 1.2511, df = 48, p-value = 0.217
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.1058851  0.4345536
sample estimates:
   cor
0.1777
```

We fail to reject the null hypothesis. There is no evidence that the correlation between petal lengths and sepal width in setosa irises differs from 0.

Nonlinear data



Nonlinear data



```
> cor.test(x, y)

Pearson's product-moment correlation

data:  x and y
t = 13.089, df = 48, p-value < 2.2e-16
alternative hypothesis: true correlation is
      not equal to 0
95 percent confidence interval:
 0.8030401 0.9327180
sample estimates:
      cor
0.8838302
```

Spearman rank nonparametric correlation

Assumes data is *monotonic* (ordinal)

```
> cor.test(x, y, method = "spearman" )

Spearman's rank correlation rho

data:  x and y
S = 0, p-value < 2.2e-16
alternative hypothesis: true rho is not equal to 0
sample estimates:
rho
  1
```

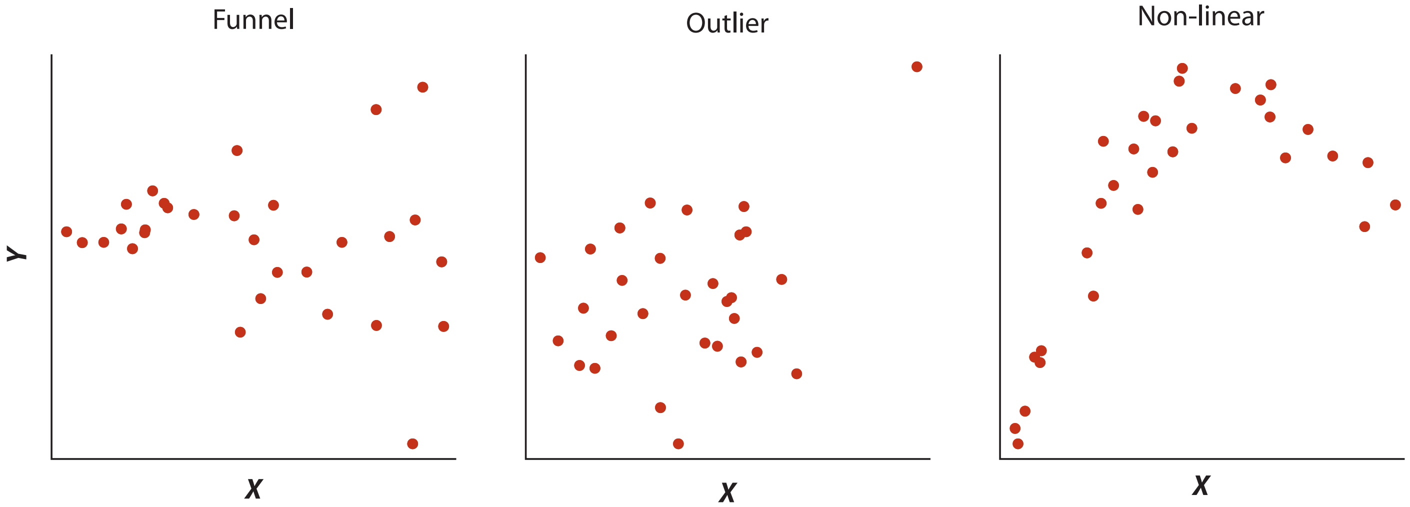
Assumptions: Check by plotting

Data are linearly related without any severe outliers

Both X and Y are normally distributed

- Robust to large N

Cloud of points is not "funnel-shaped" (fans out at end(s))



Exercise break

Regression

The simplest type of *linear model*

Predicts the value of one numeric variable from another via "line of best fit"

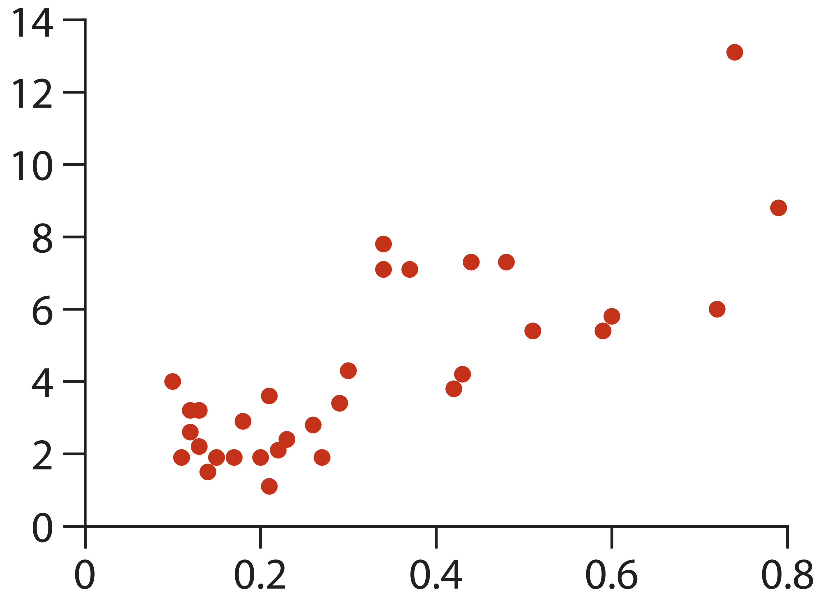
$$Y = a + bX$$

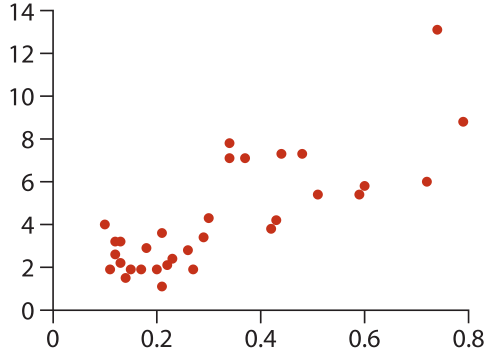
$$Y = \beta_o + \beta_1 X + \mathcal{E}$$

Residuals: $\mathcal{E}$ is a random error component that measures how far above/below the line the **actual** value of Y for a given X lies. Mean is 0.

Least squares approach

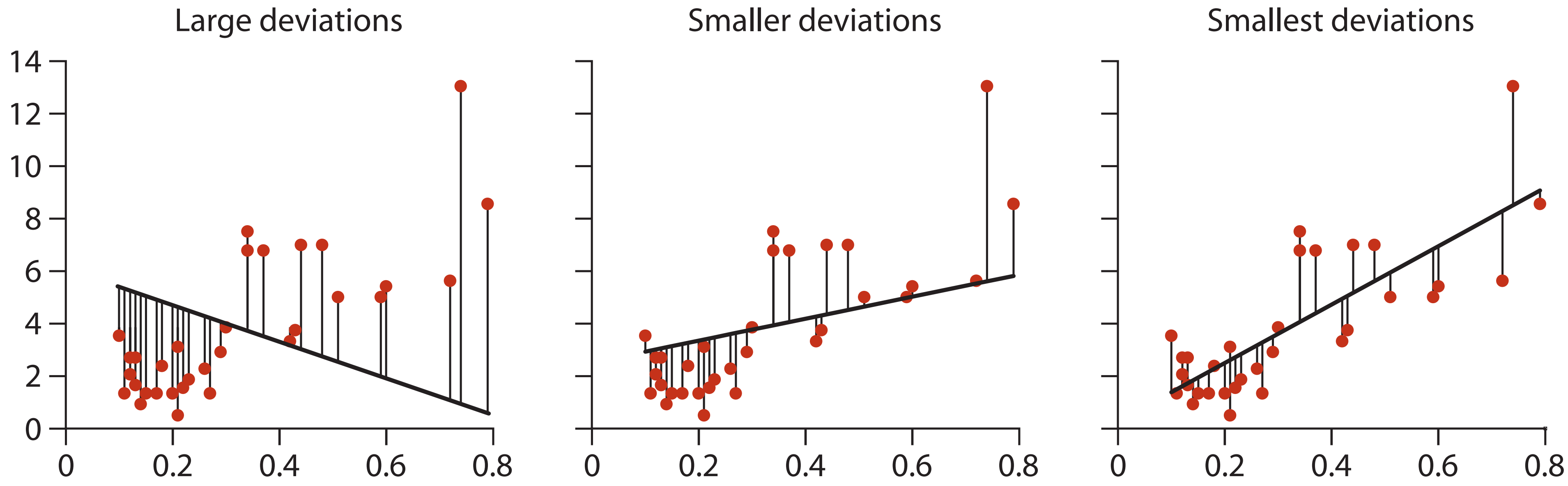
Find the line which **minimizes** the sum of squared residuals





Least squares approach

Find the line which **minimizes** the sum of squared residuals



Calculating slope and intercept

$\boldsymbol{Y = a + bX}$

$$\boldsymbol{b = \frac{cov(X,Y)}{s_X^2} = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\frac{1}{n-1}\sum(X_i - \bar{X})^2}}$$

$$\boldsymbol{a = \bar{Y} \; - b\bar{X}}$$

The point $(\bar{X}, \bar{Y})$ always goes through the regression line

Executing a linear model

```
### lm(Y ~ X, data = <data frame>) ###

> lm(Sepal.Length ~ Sepal.Width, data = setosa)


Call:
lm(formula = Sepal.Length ~ Sepal.Width, data = setosa)


Coefficients:
(Intercept)  Petal.Width
     2.6390       0.6905
```

$$\boxed{Y = 2.64 + 0.69X}$$

Testing a linear model

```
> summary( lm(Sepal.Length ~ Sepal.Width, data = setosa) )
Call:
lm(formula = Sepal.Length ~ Sepal.Width, data = setosa)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.52476 -0.16286  0.02166  0.13833  0.44428 
```

Five number summary of the distribution of residuals

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   2.6390     0.3100   8.513 3.74e-11 ***
Sepal.Width   0.6905     0.0899   7.681 6.71e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2385 on 48 degrees of freedom
Multiple R-squared:  0.5514, Adjusted R-squared:   0.542 
F-statistic: 58.99 on 1 and 48 DF,  p-value: 6.71e-10
```

SE of $\mathcal{E}$

Test for model improvement over slope=0

Coefficients

```
            Y               X
lm(Sepal.Length ~ Sepal.Width)
```

Test for null hypothesis that coefficient != 0

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   2.6390     0.3100   8.513 3.74e-11 ***
Sepal.Width   0.6905     0.0899   7.681 6.71e-10 ***
```

Expected value of Y when X=0

Expected unit increase in Y for every 1 unit increase in X

What can we conclude?

On average, setosa Sepal Length **(Y)** increases by 0.6905 cm (+/-0.0899 SE) for every 1 cm of Sepal Width **(X)**.

[If P < alpha, don't conclude this..]

R^2

R^2 is the percent of variation in Y than can be explained by X

- $0 \le R^2 \le 1$

```
Multiple R-squared:  0.5514, Adjusted R-squared:  0.542
```

$$\boldsymbol{R^2 = \left[\frac{cov(X,Y)}{s_X s_Y}\right]^2 = 1 - \frac{SS_{res}}{SS_{total}} = \frac{Explained\ variation}{Total\ variation}}$$

What can we conclude?

~55% of the variation in Setosa sepal lengths **(Y)** can be explained by Setosa sepals widths **(X).**

[If P < alpha, don't conclude this..]

Broom to the rescue

```
> summary( lm(Sepal.Length ~ Sepal.Width, data = setosa) )
Call:
lm(formula = Sepal.Length ~ Sepal.Width, data = setosa)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.52476 -0.16286  0.02166  0.13833  0.44428 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   2.6390     0.3100   8.513 3.74e-11 ***
Sepal.Width   0.6905     0.0899   7.681 6.71e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2385 on 48 degrees of freedom
Multiple R-squared:  0.5514, Adjusted R-squared:  0.542 
F-statistic: 58.99 on 1 and 48 DF,  p-value: 6.71e-10
```

Broom to the rescue

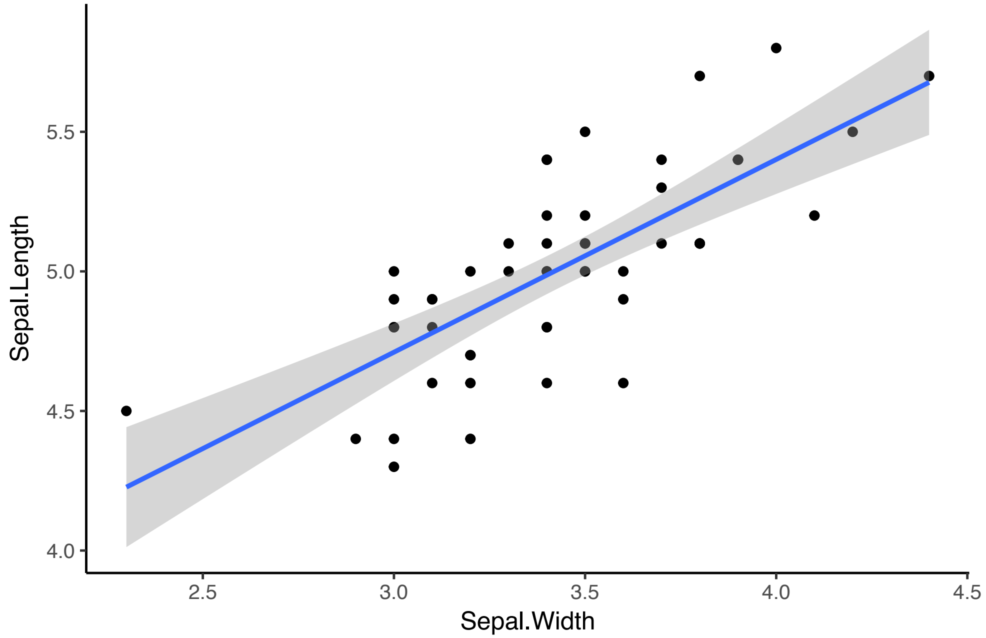
```
> library(broom)
> model <-  lm(Sepal.Length ~ Sepal.Width, data = setosa)

##### Coefficients and Pvalues #####
> tidy(model)
         term  estimate  std.error statistic      p.value
1 (Intercept) 2.6390012 0.31001431  8.512514 3.742438e-11
2 Sepal.Width 0.6904897 0.08989888  7.680738 6.709843e-10

##### Concise *one row* summary #####
> glance(model)
  r.squared adj.r.squared     sigma statistic      p.value df   logLik      AIC
1 0.5513756     0.5420292 0.2385422  58.99373 6.709843e-10  2 1.734067 2.531865
       BIC deviance df.residual
1 8.267934 2.731315          48
```

Broom to the rescue

```
##### Add columns from fit to the original data that was modeled #####
> augment(model)
Sepal.Length Sepal.Width  .fitted    .se.fit      .resid       .hat .sigma      .cooksd  .std.resid

1            5.1         3.5 5.055715 0.03435031  0.04428474 0.02073628 0.2409782 3.726311e-04  0.18760265
2            4.9         3.0 4.710470 0.05117134  0.18952960 0.04601750 0.2393991 1.596010e-02  0.81347001
3            4.7         3.2 4.848568 0.03947370 -0.14856834 0.02738325 0.2400630 5.614273e-03 -0.63152438
4            4.6         3.1 4.779519 0.04480537 -0.17951937 0.03528008 0.2395878 1.073468e-02 -0.76620575
5            5.0         3.6 5.124764 0.03710984 -0.12476423 0.02420180 0.2403616 3.476539e-03 -0.52947419
6            5.4         3.9 5.331911 0.05420835  0.06808885 0.05164186 0.2408507 2.339099e-03  0.29310589
```

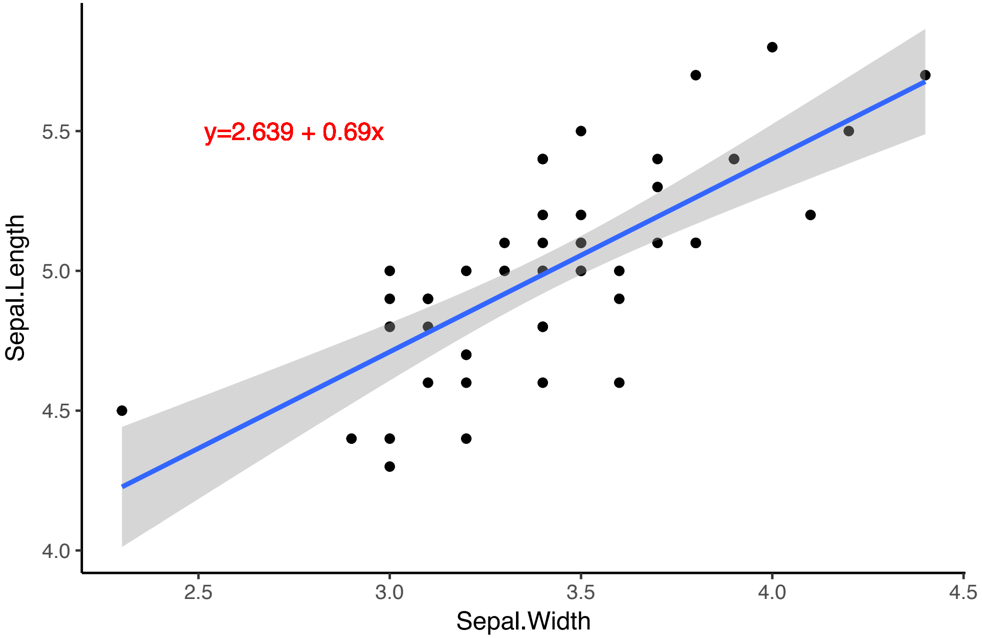
Visualizing the regression

```
ggplot(setosa, aes(x = Sepal.Width, y =  Sepal.Length)) +
      geom_point() + geom_smooth(method = "lm")
```



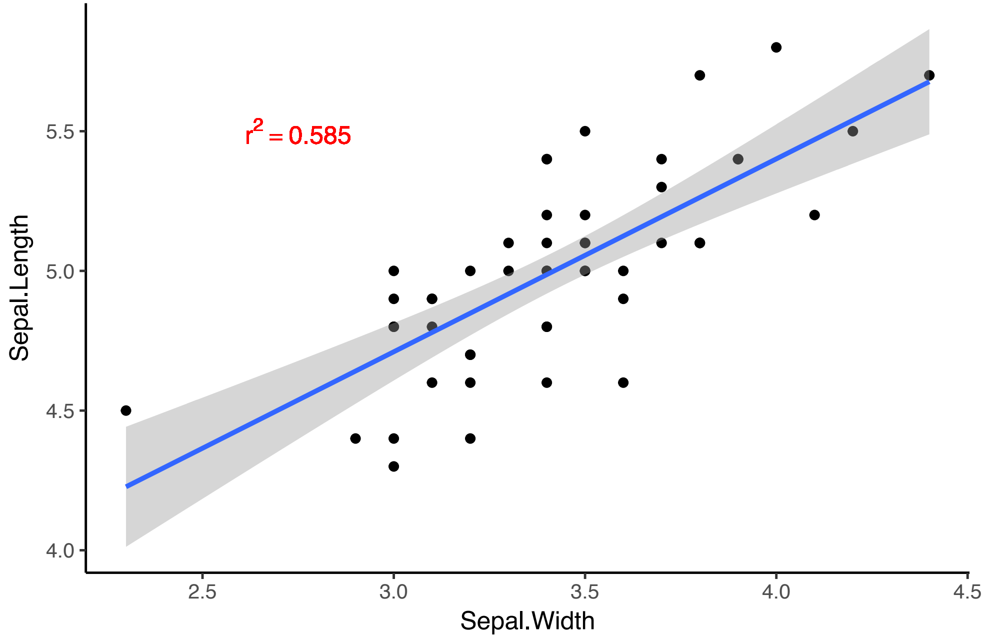
Visualizing the regression

```
ggplot(setosa, aes(x = Sepal.Width, y =  Sepal.Length)) +
      geom_point() + geom_smooth(method = "lm") +
      geom_text(x = 2.75, y = 5.5, label = "y=2.639 + 0.69x", color="red")
```



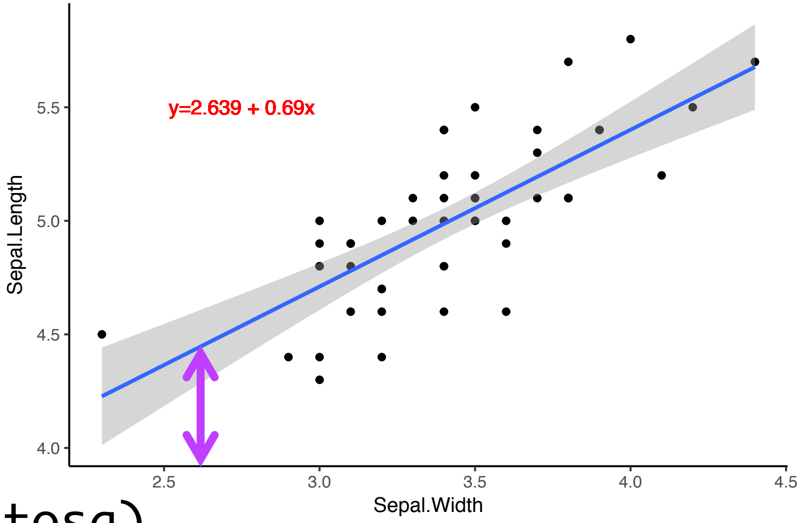
Visualizing the regression

```
ggplot(setosa, aes(x = Sepal.Width, y =  Sepal.Length)) +
        geom_point() + geom_smooth(method = "lm") +
         geom_text(x=2.75, y=5.5, label="r^2 == 0.585", parse=TRUE, color="red")
```



Using the model: Predicting responses

What is sepal length for a sepal width of 2.6?



```
> model <- lm(Sepal.Length ~ Sepal.Width, data = setosa)

> new.data <-tibble(Sepal.Width = 2.6) ## Same column name as model's predictor

> predict(model, new.data)
       1
4.434275
```

Predicting with intervals

Confidence interval

- Range that is likely to contain **the mean response**

$$\hat{y}_h \pm t_{(\alpha/2,n-2)} \times \sqrt{MSE\left(\frac{1}{n} + \frac{(x_h - \bar{x})^2}{\sum(x_i - \bar{x})^2}\right)}$$

Prediction interval

- Range that is likely to contain **the response value of a single new observation**
- Wider than CI due to added uncertainty for predicting a single point

$$\hat{y}_h \pm t_{(\alpha/2,n-2)} \times \sqrt{MSE \times \left(1 + \frac{1}{n} + \frac{(x_h - \bar{x})^2}{\sum(x_i - \bar{x})^2}\right)}$$

Predicting with intervals

```
> predict(model, new.data)
       1 
4.434275 

> predict(model, new.data, interval = "confidence")
       fit      lwr      upr
1 4.434275 4.269957 4.598592

> predict(model, new.data, interval = "predict")
       fit      lwr      upr
1 4.434275 3.927287 4.941262

> predict(model, new.data, interval = "confidence", level = 0.9)
       fit      lwr      upr
1 4.434275 4.297205 4.571344
```

Assumptions of linear models

Residuals are normally distributed

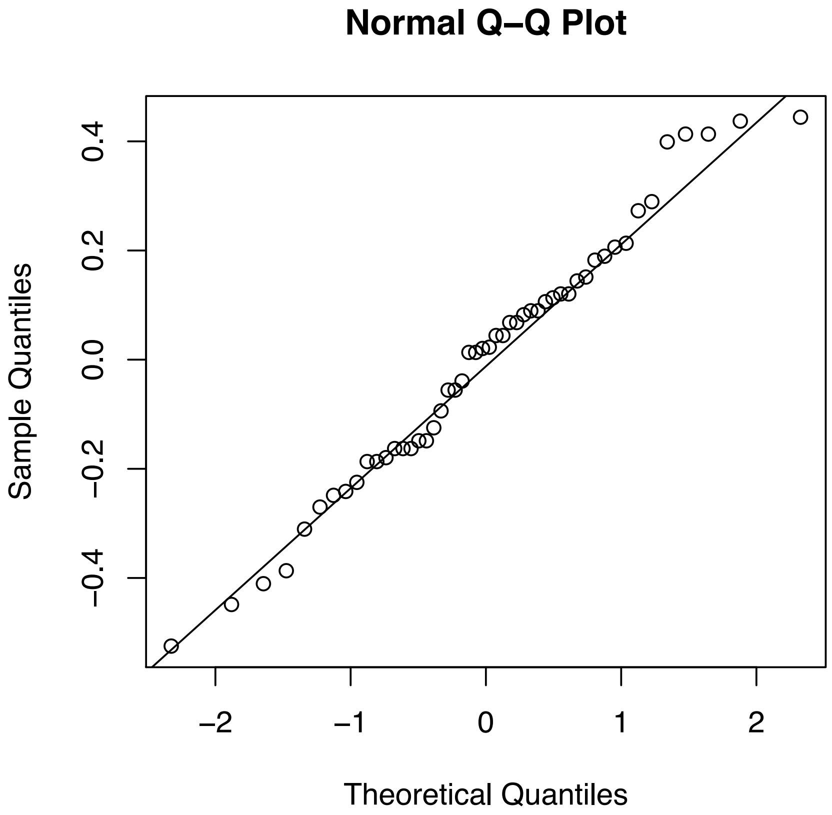
The variance is the same for all predictors*

Predictors are independent of each other*

The relationship between response and any numeric predictors is linear*

Normality of residuals

```
> augmented <- augment(model)
> qqnorm(augmented$.resid)
> qqline(augmented$.resid)
```



How to check regression assumptions

1. **Plot response and predictor against each other to ensure linearity**
   - **Critically important**
2. Plot the residuals to ensure normality
   - Important, usually overlooked
   - Most times we are robust to departures

Exercise break

Linear Models

```
lm(Numeric response ~ <predictors>)
```

Linear Models

```
lm(Numeric response ~ <predictors>)
```

Single numeric predictor: Regression

Single categorical predictor: ANOVA

Multiple numeric predictors: multiple regression

Multiple categorical predictors: *n*-way ANOVA

Single categorical and *n* numeric predictors: ANCOVA

Multiple categorical and *n* numeric predictors: linear model

ANOVA meets linear modeling

```
        group calcium
1      normal    1200
2      normal    1000
3      normal     980
4      normal     900
5      normal     750
6      normal     800
7  osteopenia    1000
8  osteopenia    1100
9  osteopenia     700
10 osteopenia     800
11 osteopenia     500
12 osteopenia     700
...
```

```
> summary(aov(calcium ~ group, data = tidy.data))
            Df Sum Sq Mean Sq F value   Pr(>F)
group        2 944144  472072   14.36 0.000328 ***
Residuals   15 493167   32878
```

$$\frac{944144}{944144 + 493167} = 0.656$$

```
> summary(lm(calcium ~ group, data = tidy.data))

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)         938.33      74.02  12.676 2.04e-09 ***
grouposteopenia    -138.33     104.69  -1.321 0.206168
grouposteoporosis  -540.00     104.69  -5.158 0.000117 ***

Residual standard error: 181.3 on 15 degrees of freedom
Multiple R-squared:  0.6569, Adjusted R-squared:  0.6111
F-statistic: 14.36 on 2 and 15 DF,  p-value: 0.000328
```

On average, the normal group consumes 938.33 calcium

~~**Compared to the normal group, the osteopenia group consumes on average -138.33 less calcium.**~~

Compared to the normal group, the osteoporosis group consumes on average -540 less calcium.

Which group you belong to explains ~66% of the variation in calcium intake

Briefly, bootstrapping the regression

```
> library(slipper)
> setosa %>%
    slipper_lm(Sepal.Length ~ Sepal.Width, B=1e3)%>% head()
         term     value      type
1 (Intercept) 2.6390012  observed
2 Sepal.Width 0.6904897  observed
3 (Intercept) 2.0900929 bootstrap
4 Sepal.Width 0.8467474 bootstrap
5 (Intercept) 2.7629316 bootstrap
6 Sepal.Width 0.6527575 bootstrap

setosa %>%
    slipper_lm(Sepal.Length ~ Sepal.Width, B=1e3) %>%
    filter(type == "bootstrap", term == "Sepal.Width") %>%
    summarize(mean = mean(value),
              ci_low = quantile(value,0.025),
              ci_high = quantile(value,0.975))
       mean    ci_low   ci_high
1 0.6945918 0.5302098 0.8961058
```