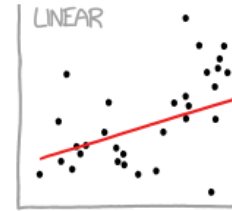


ANOVAs and Generalized Linear Models

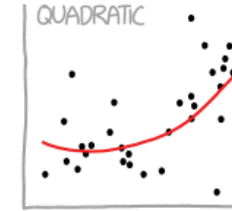
ANOVAs
T-tests
Binomial Test
Chi-Square Test

All can be done as GLMs

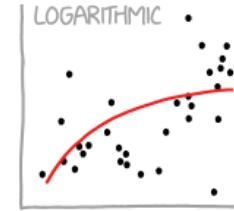
CURVE-FITTING METHODS AND THE MESSAGES THEY SEND



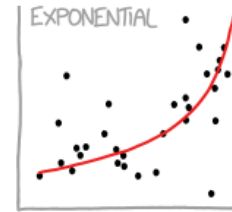
"HEY, I DID A
REGRESSION."



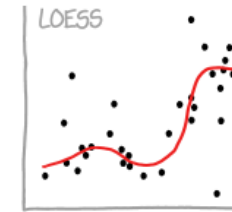
"I WANTED A CURVED
LINE, SO I MADE ONE
WITH MATH."



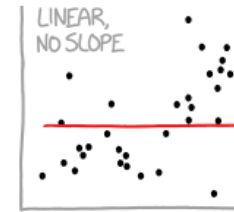
"LOOK, IT'S
TAPERING OFF!"



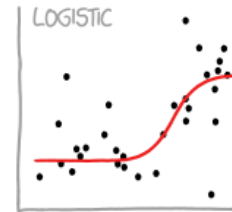
"LOOK, IT'S GROWING
UNCONTROLLABLY!"



"I'M SOPHISTICATED, NOT
LIKE THOSE BUMBLING
POLYNOMIAL PEOPLE."



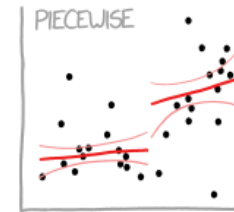
"I'M MAKING A
SCATTER PLOT BUT
I DON'T WANT TO."



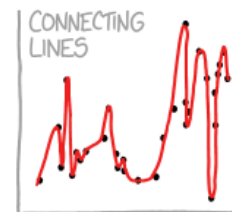
"I NEED TO CONNECT THESE
TWO LINES, BUT MY FIRST IDEA
DIDN'T HAVE ENOUGH MATH."



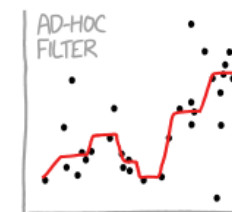
"LISTEN, SCIENCE IS HARD.
BUT I'M A SERIOUS
PERSON DOING MY BEST."



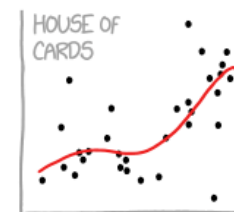
"I HAVE A THEORY,
AND THIS IS THE ONLY
DATA I COULD FIND."




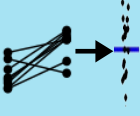
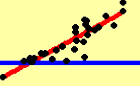
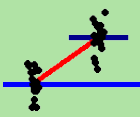
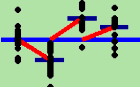
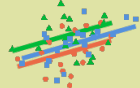
"I CLICKED 'SMOOTH
LINES' IN EXCEL."



"I HAD AN IDEA FOR HOW
TO CLEAN UP THE DATA.
WHAT DO YOU THINK?"



"AS YOU CAN SEE, THIS
MODEL SMOOTHLY FITS
THE- WAIT NO NO DON'T
EXTEND IT AAAAAA!!!"

| | Common name | Built-in function in R | Equivalent linear model in R | Exact? | The linear model in words | Icon |
|--|---|--|---|--|---|---|
| Simple regression: $\text{lm}(y \sim 1 + x)$ | y is independent of x P: One-sample t-test N: Wilcoxon signed-rank | t.test(y) wilcox.test(y) | $\text{lm}(y \sim 1)$ $\text{lm}(\text{signed_rank}(y) \sim 1)$ | ✓ for N >14 | One number (intercept, i.e., the mean) predicts y . - (Same, but it predicts the <i>signed rank</i> of y .) |  |
| | P: Paired-sample t-test N: Wilcoxon matched pairs | t.test(y1, y2, paired=TRUE) wilcox.test(y1, y2, paired=TRUE) | $\text{lm}(y_2 - y_1 \sim 1)$ $\text{lm}(\text{signed_rank}(y_2 - y_1) \sim 1)$ | ✓ for N >14 | One intercept predicts the pairwise y₂-y₁ differences. - (Same, but it predicts the <i>signed rank</i> of y₂-y₁ .) |  |
| | y ~ continuous x P: Pearson correlation N: Spearman correlation | cor.test(x, y, method='Pearson') cor.test(x, y, method='Spearman') | $\text{lm}(y \sim 1 + x)$ $\text{lm}(\text{rank}(y) \sim 1 + \text{rank}(x))$ | ✓ for N >10 | One intercept plus x multiplied by a number (slope) predicts y . - (Same, but with <i>ranked x</i> and y) |  |
| | y ~ discrete x P: Two-sample t-test P: Welch's t-test N: Mann-Whitney U | t.test(y1, y2, var.equal=TRUE) t.test(y1, y2, var.equal=FALSE) wilcox.test(y1, y2) | $\text{lm}(y \sim 1 + G_2)^A$ $\text{gls}(y \sim 1 + G_2, \text{weights}=\dots^B)^A$ $\text{lm}(\text{signed_rank}(y) \sim 1 + G_2)^A$ | ✓ ✓ for N >11 | An intercept for group 1 (plus a difference if group 2) predicts y . - (Same, but with one variance <i>per group</i> instead of one common.) - (Same, but it predicts the <i>signed rank</i> of y .) |  |
| Multiple regression: $\text{lm}(y \sim 1 + x_1 + x_2 + \dots)$ | P: One-way ANOVA N: Kruskal-Wallis | aov(y ~ group) kruskal.test(y ~ group) | $\text{lm}(y \sim 1 + G_2 + G_3 + \dots + G_N)^A$ $\text{lm}(\text{rank}(y) \sim 1 + G_2 + G_3 + \dots + G_N)^A$ | ✓ for N >11 | An intercept for group 1 (plus a difference if group $\neq 1$) predicts y . - (Same, but it predicts the <i>rank</i> of y .) |  |
| | P: One-way ANCOVA | aov(y ~ group + x) | $\text{lm}(y \sim 1 + G_2 + G_3 + \dots + G_N + x)^A$ | ✓ | - (Same, but plus a slope on x .) <i>Note: this is discrete AND continuous. ANCOVAs are ANOVAs with a continuous x.</i> |  |
| | P: Two-way ANOVA | aov(y ~ group * sex) | $\text{lm}(y \sim 1 + G_2 + G_3 + \dots + G_N + S_2 + S_3 + \dots + S_K + G_2*S_2 + G_3*S_3 + \dots + G_N*S_K)$ | ✓ | Interaction term: changing sex changes the y ~ group parameters. <i>Note: $G_{2 \text{ to } N}$ is an indicator (0 or 1) for each non-intercept levels of the group variable. Similarly for $S_{2 \text{ to } K}$ for sex. The first line (with G_i) is main effect of group, the second (with S_j) for sex and the third is the group * sex interaction. For two levels (e.g. male/female), line 2 would just be "S_2" and line 3 would be S_2 multiplied with each G_i.</i> | [Coming] |
| | Counts ~ discrete x N: Chi-square test | chisq.test(groupXsex_table) | Equivalent log-linear model $\text{glm}(y \sim 1 + G_2 + G_3 + \dots + G_N + S_2 + S_3 + \dots + S_K + G_2*S_2 + G_3*S_3 + \dots + G_N*S_K, \text{family}=\dots)^A$ | ✓ | Interaction term: (Same as Two-way ANOVA.) <i>Note: Run glm using the following arguments: <code>glm(model, family=poisson())</code> As linear-model, the Chi-square test is $\log(y_i) = \log(N) + \log(\alpha_i) + \log(\beta_j) + \log(\alpha_i\beta_j)$ where α_i and β_j are proportions. See more info in the accompanying notebook.</i> | Same as Two-way ANOVA |
| | N: Goodness of fit | chisq.test(y) | $\text{glm}(y \sim 1 + G_2 + G_3 + \dots + G_N, \text{family}=\dots)^A$ | ✓ | (Same as One-way ANOVA and see Chi-Square note.) | 1W-ANOVA |

Stats and Genetics/Evolution

The Correlation between relatives on the supposition of Mendelian Inheritance

By R. A. FISHER, B.A.

Communicated by Professor J. ARTHUR THOMSON

With Four Figures in Text

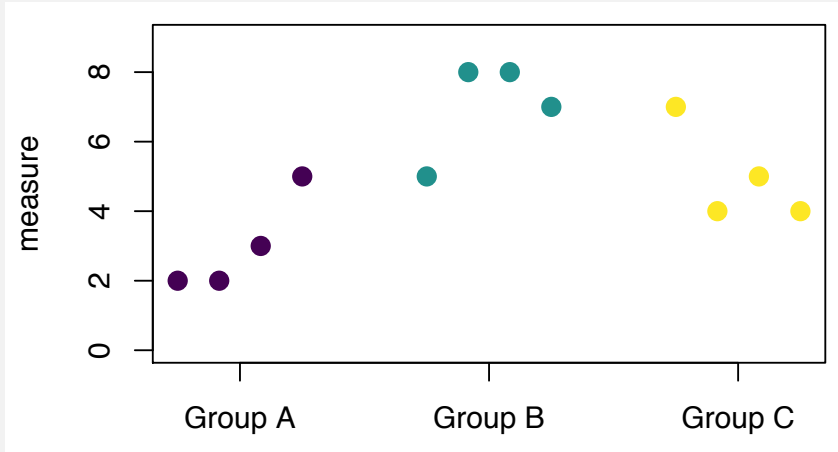
(MS. received 15 June 1918. Read 8 July 1918. Issued separately 1 October 1918)

Analysis of Variance

- Used to compare the means among more than two groups
- If you are comparing three groups, for instance, you cannot just do three pair-wise t -tests – this approach would cause too many false positives
- ANOVA takes into account the fact that you are comparing multiple groups and controls the false positive rate.

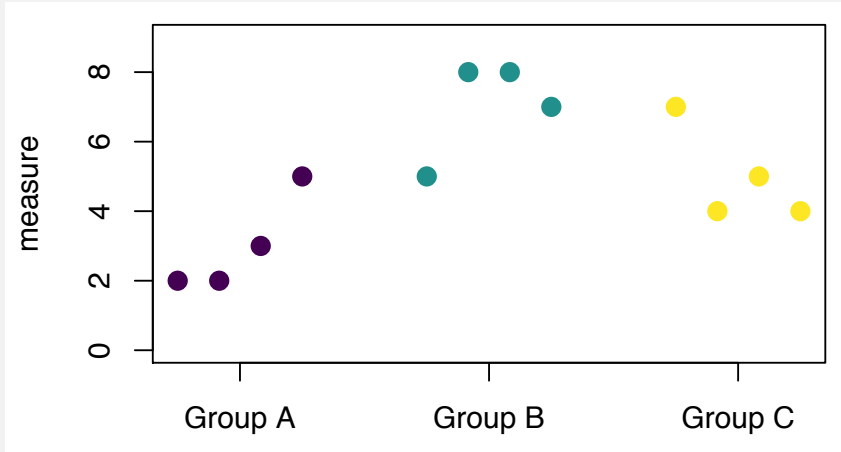
Analysis of Variance

| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



Analysis of Variance

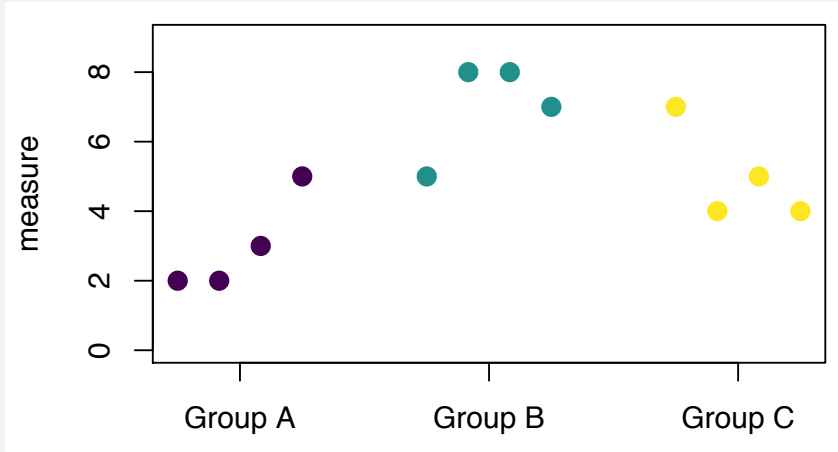
| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



$$f \text{ statistic} = \frac{\frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

Analysis of Variance

| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



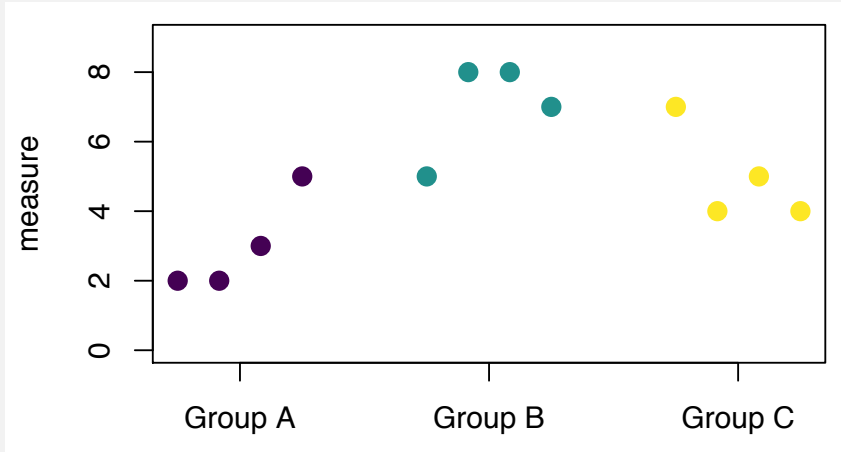
$$f \text{ statistic} = \frac{\frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

number of groups -1

number of sample – number of groups

Analysis of Variance

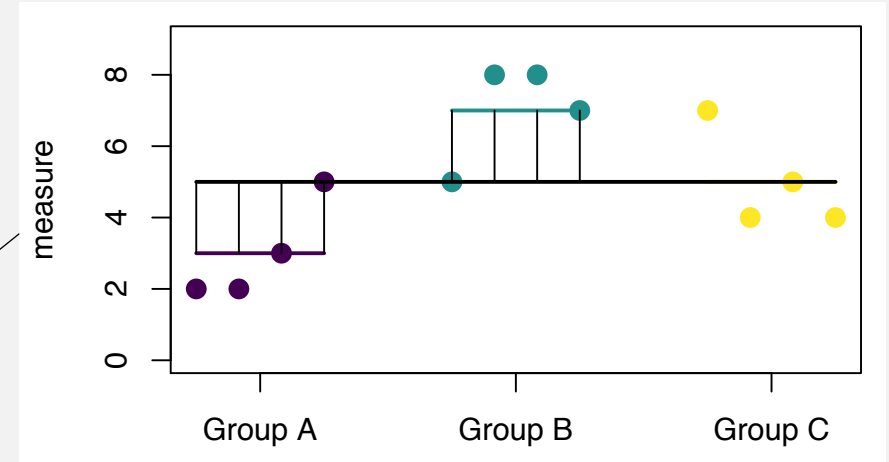
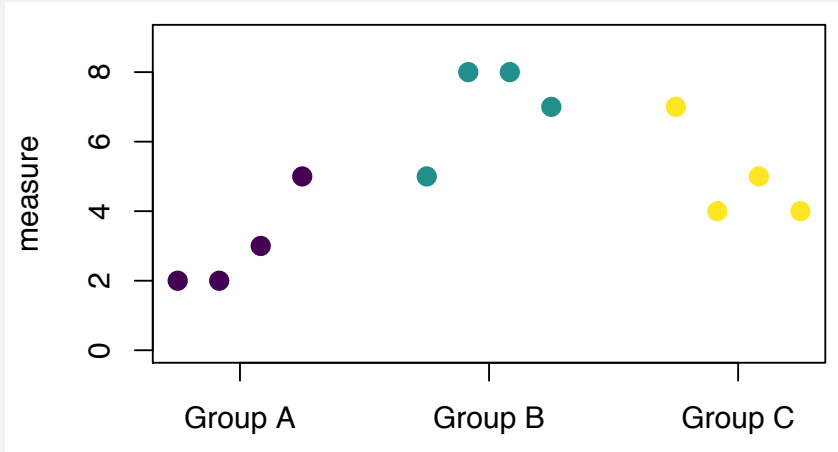
| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



$$f \text{ statistic} = \frac{\frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

Analysis of Variance

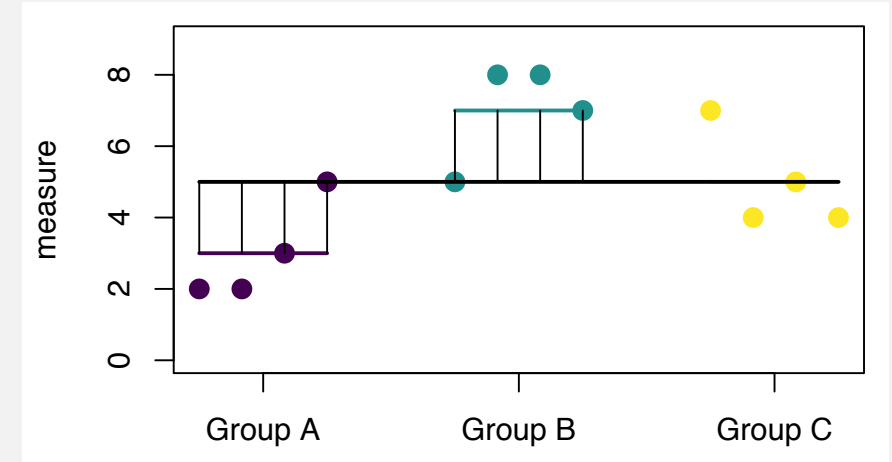
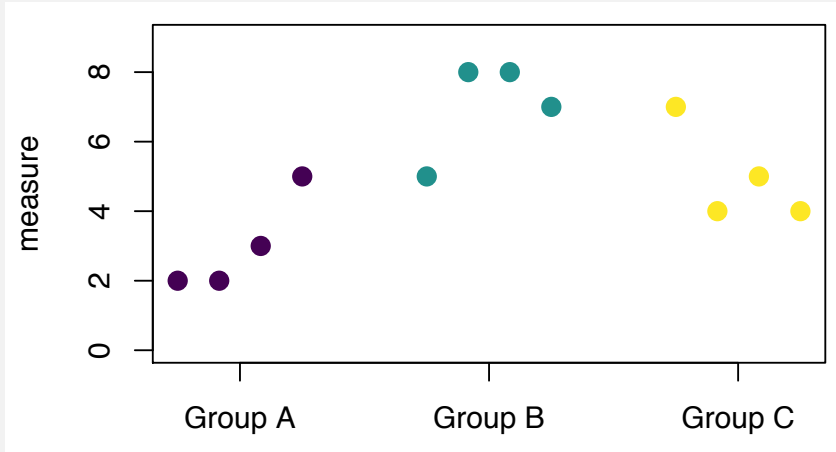
| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



$$f \text{ statistic} = \frac{\frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

Analysis of Variance

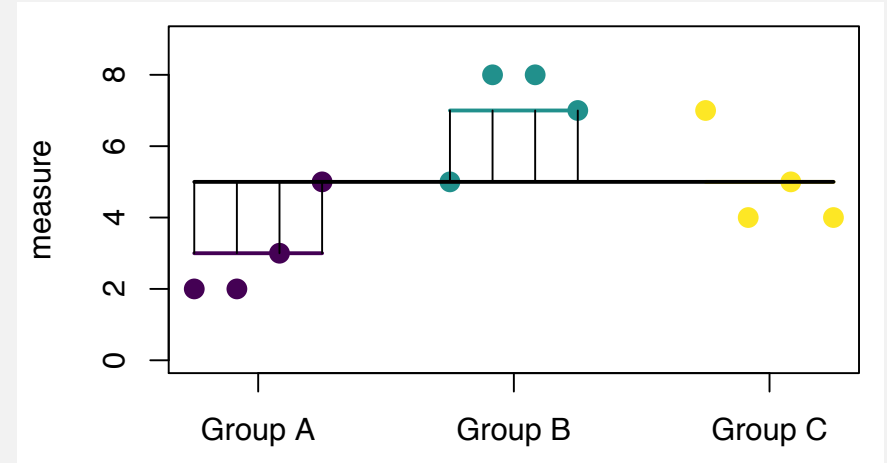
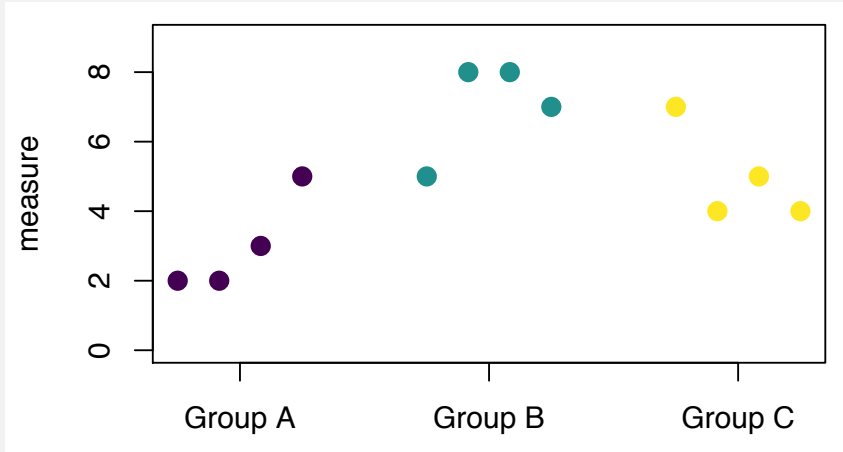
| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



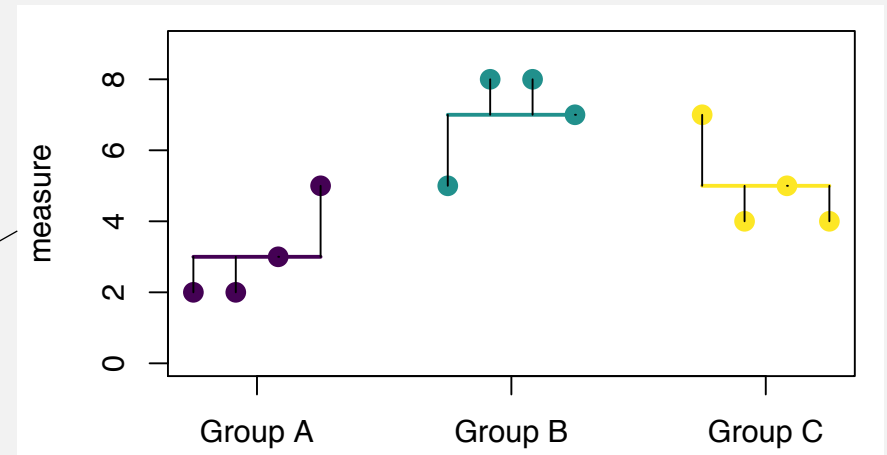
$$f \text{ statistic} = \frac{\frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

Analysis of Variance

| A | B | C |
|---|---|---|
| 2 | 5 | 7 |
| 2 | 8 | 4 |
| 3 | 8 | 5 |
| 5 | 7 | 4 |



$$f \text{ statistic} = \frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}} \div \frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}$$

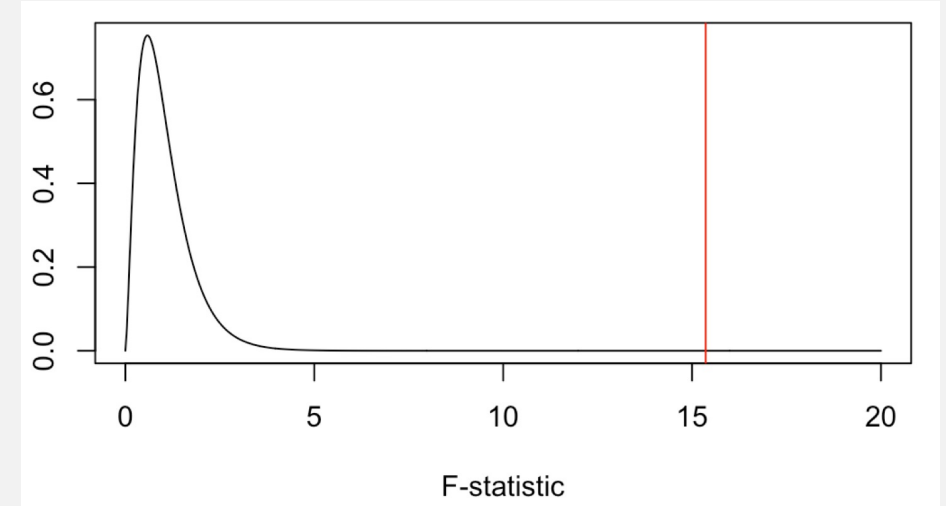


Running ANOVA in R

```
> data("chickwts")
> fit <- aov(weight~feed, data=chickwts)
> summary(fit)
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|----|--------|---------|---------|--------------|
| feed | 5 | 231129 | 46226 | 15.37 | 5.94e-10 *** |
| Residuals | 65 | 195556 | 3009 | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



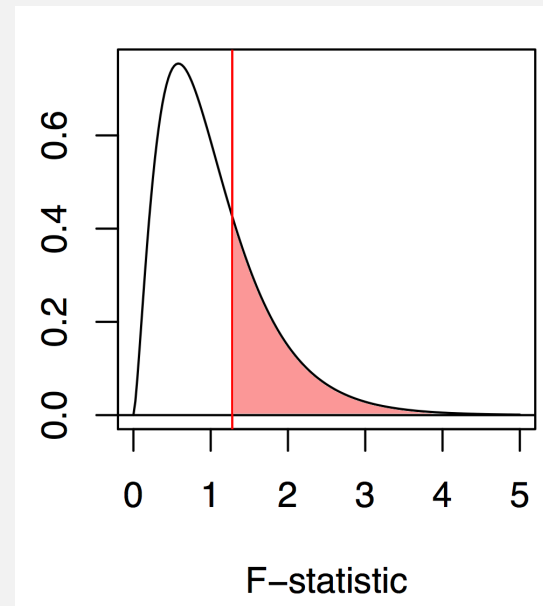
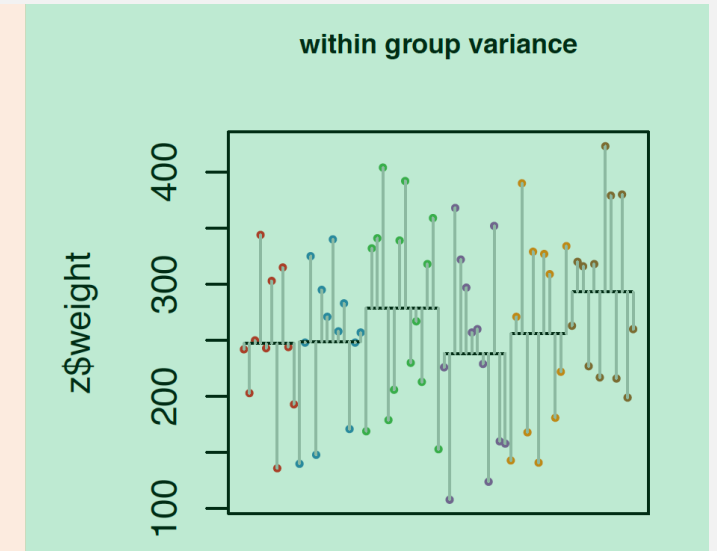
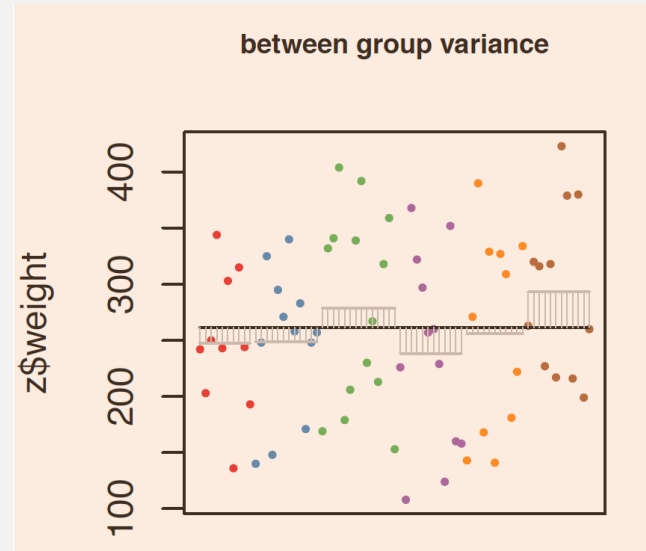
This significant result tells us that at least one of the groups of chickens have significantly different mean weights than at least one other groups. (significant ANOVA result allows us to reject the null that they are all the same)

$$f \text{ statistic} = \frac{\sum_{j=1}^p n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}} \div \frac{\sum_{j=1}^p \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}$$

Running ANOVA in R

```
> data("chickwts")
> z <- chickwts
> z$weight <- sample(z$weight)
> fit <- aov(weight~feed, data=z)
> summary(fit)
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|----|--------|---------|---------|--------|
| feed | 5 | 32244 | 6449 | 1.063 | 0.389 |
| Residuals | 65 | 394441 | 6068 | | |



Post-hoc tests

If your ANOVA is significant, you may be interested in discovering which groups are different from one another

A variety of post-hoc comparisons of the means can be used

Fisher's LSD

- Least conservative test, basically uses t -tests to compare the means

Scheffe's method

- Performs all comparisons simultaneously, but has relatively low power

Tukey-Kramer method

- A pair-wise method, like a t -test, but corrected for multiple comparisons

Post-hoc tests

```
> data("chickwts")
> fit <- aov(weight~feed, data=chickwts)
> TukeyHSD(fit)
```



```
Tukey multiple comparisons of means
 95% family-wise confidence level
```

```
Fit: aov(formula = weight ~ feed, data = chickwts)
```

```
$feed
```

| | diff | lwr | upr | p adj |
|---------------------|-------------|-------------|-----------|-----------|
| horsebean-casein | -163.383333 | -232.346876 | -94.41979 | 0.0000000 |
| linseed-casein | -104.833333 | -170.587491 | -39.07918 | 0.0002100 |
| meatmeal-casein | -46.674242 | -113.906207 | 20.55772 | 0.3324584 |
| soybean-casein | -77.154762 | -140.517054 | -13.79247 | 0.0083653 |
| sunflower-casein | 5.333333 | -60.420825 | 71.08749 | 0.9998902 |
| linseed-horsebean | 58.550000 | -10.413543 | 127.51354 | 0.1413329 |
| meatmeal-horsebean | 116.709091 | 46.335105 | 187.08308 | 0.0001062 |
| soybean-horsebean | 86.228571 | 19.541684 | 152.91546 | 0.0042167 |
| sunflower-horsebean | 168.716667 | 99.753124 | 237.68021 | 0.0000000 |
| meatmeal-linseed | 58.159091 | -9.072873 | 125.39106 | 0.1276965 |
| soybean-linseed | 27.678571 | -35.683721 | 91.04086 | 0.7932853 |
| sunflower-linseed | 110.166667 | 44.412509 | 175.92082 | 0.0000884 |
| soybean-meatmeal | -30.480519 | -95.375109 | 34.41407 | 0.7391356 |
| sunflower-meatmeal | 52.007576 | -15.224388 | 119.23954 | 0.2206962 |
| sunflower-soybean | 82.488095 | 19.125803 | 145.85039 | 0.0038845 |

anova and aov functions will both perform an ANOVA but the results are stored slightly differently. For this posthoc test we want the aov format

Interpreting post-hoc tests

```
> data("chickwts")
> fit <- aov(weight~feed, data=chickwts)
> TukeyHSD(fit)
  Tukey multiple comparisons of means
    95% family-wise confidence level
```

```
Fit: aov(formula = weight ~ feed, data = chickwts)
```

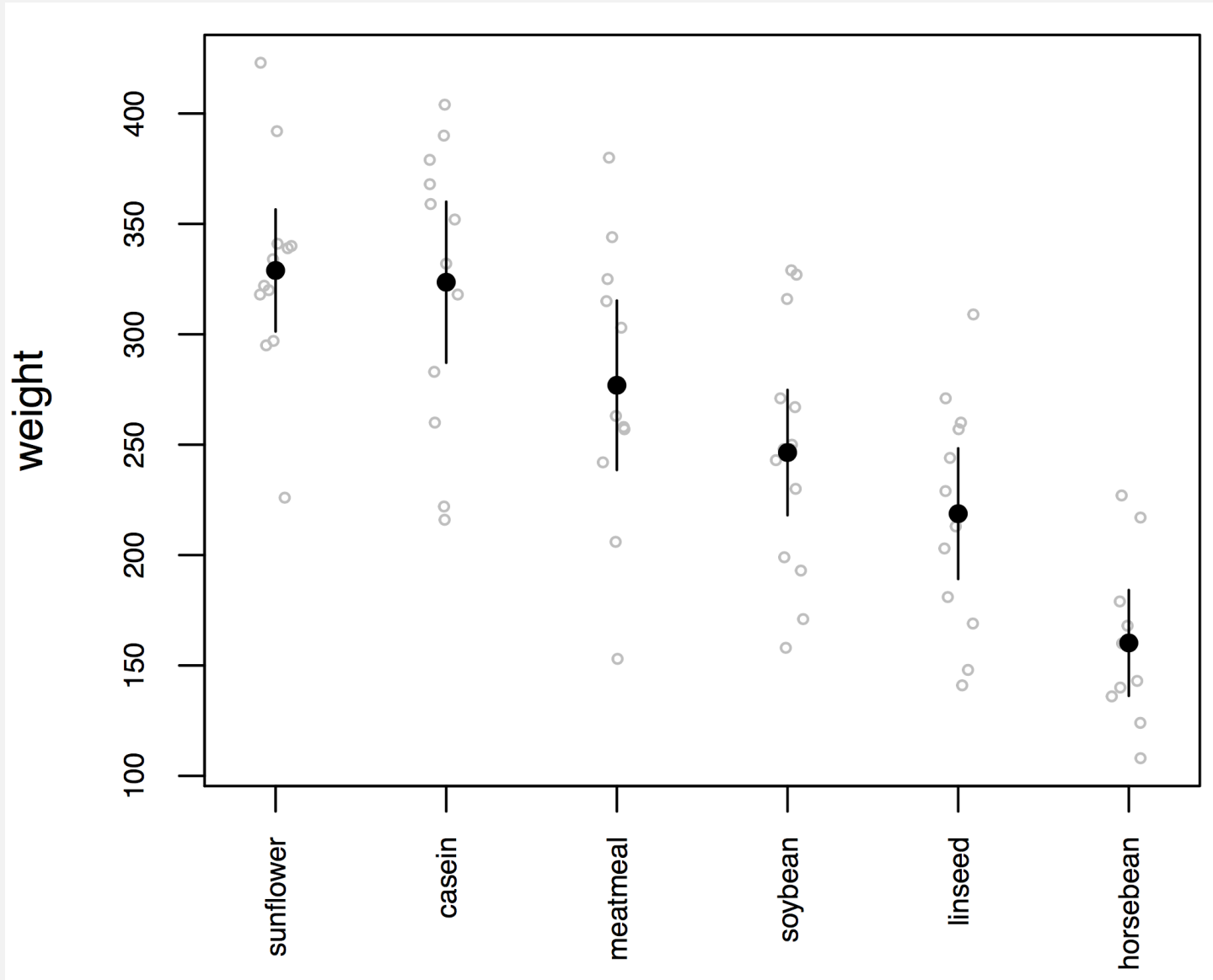
```
$feed
```

| | diff | lwr | upr | p adj |
|---------------------|-------------|-------------|-----------|-----------|
| horsebean-casein | -163.383333 | -232.346876 | -94.41979 | 0.0000000 |
| linseed-casein | -104.833333 | -170.587491 | -39.07918 | 0.0002100 |
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| sunflower-soybean | 82.488095 | 19.125803 | 145.85039 | 0.0038845 |

When we examine all the significantly different ones we can draw several conclusions:

- 1) Chicks fed casein are significantly heavier than those fed horsebean, linseed, and soybean.
- 2) Chicks fed horsebean are significantly lighter than those fed meatmeal, soybean, and sunflower.
- 3) Chicks fed sunflower are significantly heavier than those fed linseed or soybean.

Plotting this kind of data



Our results from the ANOVA and Tukey match up pretty well with our rules of thumb about 95% CI overlaps

Example of code

```
#sets the order of the treatments
chickwts$feed <- factor(chickwts$feed,
                        levels=c("sunflower", "casein",
                                "meatmeal", "soybean",
                                "linseed", "horsebean"))

stripchart(weight ~ feed, data=chickwts,
            method = "jitter", vertical = TRUE, cex.axis = .7,
            col = "gray", pch = 1, cex = .5, las = 3)

#Add error bars:
#First calculate means and SDs
meanShift <- tapply(chickwts$weight, chickwts$feed, mean)
sdevShift <- tapply(chickwts$weight, chickwts$feed, sd)
n <- tapply(chickwts$weight, chickwts$feed, length)
feed_table <- data.frame(mean = meanShift,
                          std.dev = sdevShift, n = n)

#Now add the SEM for each group:
seShift <- 1.96 * sdevShift / sqrt(n)
segments(1:6, meanShift - seShift,
          1:6, meanShift + seShift)
points(meanShift ~ c(1:6), pch = 16)
```

Assumptions of the ANOVA

- The variable is normally distributed within each group
- The variance is the same in the different groups
- The design is balanced – you have the same sample size for each group
- But... ANOVA is fairly robust to violations of these assumptions

A Non-Parametric Alternative

- Kruskal-Wallis Test
- Based on ranks
- The multiple-group version of the Mann-Whitney U-test

R-implementation:

```
> kruskal.test(weight ~ feed, data = chickwts)
```

```
Kruskal-Wallis rank sum test
```

```
data: weight by feed
```

```
Kruskal-Wallis chi-squared = 37.343, df = 5, p-value = 5.113e-07
```

p-value suggests this
test has lower power
than ANOVA



A Non-Parametric post-hoc

- Dunn's test – is the non-parametric equivalent of the Tukey

Not in base R need to install:

```
install.packages("dunn.test", dependencies=TRUE)
```

```
> dunn.test(chickwts$weight, g=chickwts$feed,  
+           altp = T, method = "bonferroni")  
Kruskal-Wallis rank sum test
```

data: x and group

Kruskal-Wallis chi-squared = 37.3427, df = 5, p-value = 0

Comparison of x by group
(Bonferroni)

| Col Mean-I | | | | | |
|------------|-----------|-----------|-----------|-----------|-----------|
| Row Mean | casein | horsebea | linseed | meatmeal | soybean |
| horsebea | 4.813069 | | | | |
| | 0.0000* | | | | |
| | | | | | |
| linseed | 3.308292 | -1.658736 | | | |
| | 0.0141* | 1.0000 | | | |
| | | | | | |
| meatmeal | 1.415755 | -3.364059 | -1.819817 | | |
| | 1.0000 | 0.0115* | 1.0000 | | |
| | | | | | |
| soybean | 2.499922 | -2.602093 | -0.933255 | 0.974144 | |
| | 0.1863 | 0.1390 | 1.0000 | 1.0000 | |
| | | | | | |
| sunflowe | -0.182969 | -4.987524 | -3.491262 | -1.594703 | -2.689798 |
| | 1.0000 | 0.0000* | 0.0072* | 1.0000 | 0.1072 |

alpha = 0.05

Reject Ho if p <= alpha

ANOVA Summary

- ANOVA is the foundation of essentially all tests comparing multiple means
- Don't make it too complicated – the null hypothesis is simple: they are all the same.
- Post-hoc tests are important for determining which means are the source of a significant ANOVA.
- You can only justify a post-hoc test if the ANOVA is significant in the first place.
- Before applying ANOVA, check that your data fit the assumptions (consider transforming the data lots of times this will be based on your biological knowledge because you will have insufficient data to say much about the observed distribution)

ANOVA Practice Problems

- Use the chick weights dataset included in R data("chickwts"). Reduce the data down to just soybean and sunflower. Run an ANOVA and determine whether these foods lead to significant differences in weight.

Hypothesis testing has limits

Often times we want to say more than something has an effect. We want to understand exactly how a predictor variable impacts a response variable.

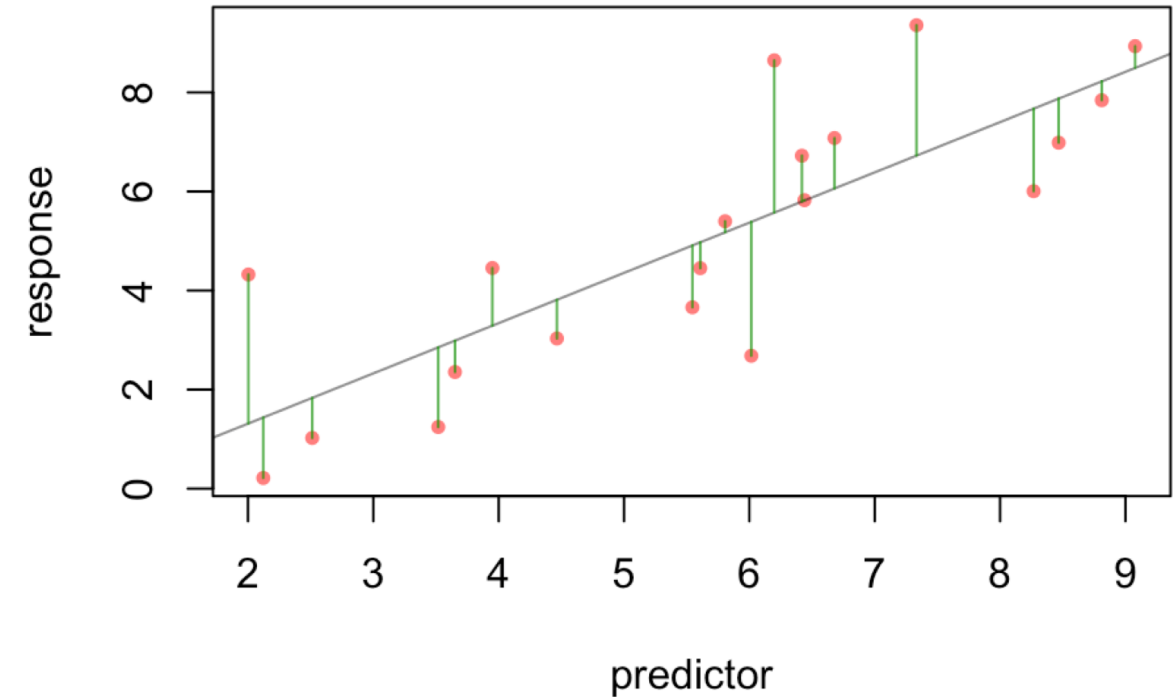
Often times we have complex relationships where several variables (continuous and discrete) impact our response variable and we need to understand how all of these things work together to determine our observations.

The Plan

1. Linear regression
2. Poisson regression
3. Binomial regression
4. GLMs with a mix of variable types
5. Mixed effects models

Regression in R

- 1) With linear regression we find the linear equation that best predicts the values of Y based on the values of X.
- 2)
$$y = bx + a$$
- 3) Least-squares regression minimizes the squared deviations of the data points from that line.



Example of regression

```
set.seed(3)
x <- runif(min = 1, max = 10, 20)
y <- rnorm(20, mean = x, sd = 2)
fit.xy <- lm(y ~ x)
summary(fit.xy)
```

$$y = bx + a$$

$$t = \frac{\beta_0}{SE_b}$$

Call:
lm(formula = y ~ x)

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|---------|---------|--------|--------|
| -2.7060 | -0.9742 | -0.4539 | 0.9479 | 3.0728 |

Coefficients:

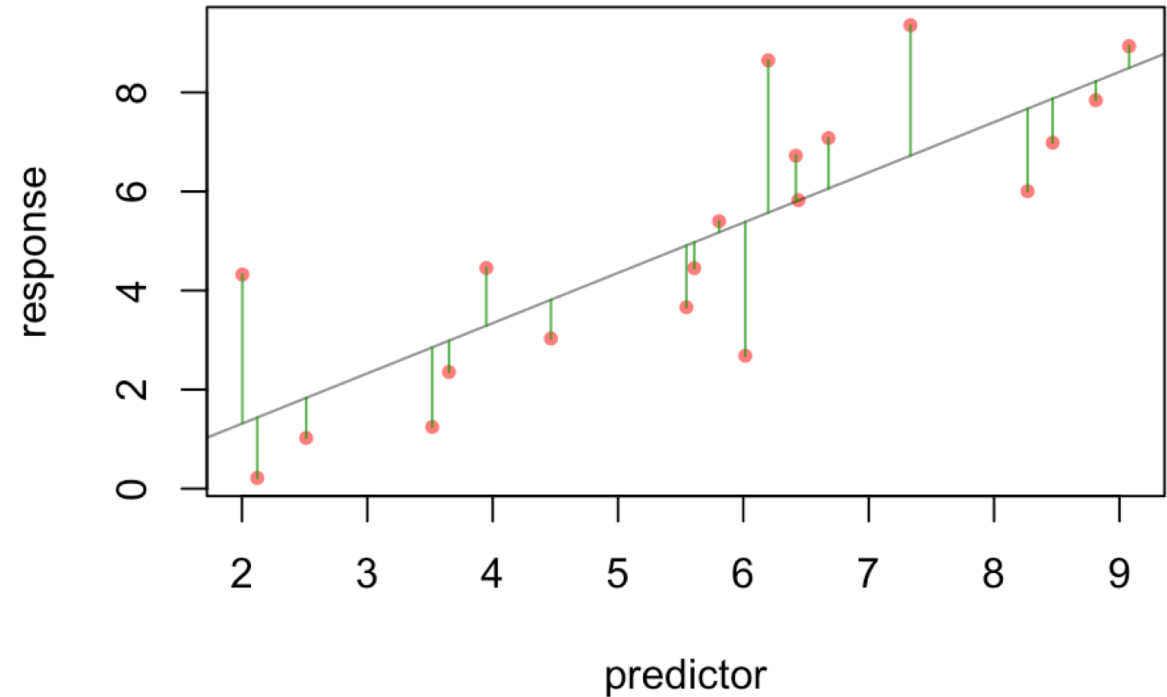
| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|----------|------------|---------|--------------|
| (Intercept) | -0.7173 | 1.0302 | -0.696 | 0.495 |
| x | 1.0150 | 0.1708 | 5.943 | 1.27e-05 *** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.625 on 18 degrees of freedom

Multiple R-squared: 0.6624, Adjusted R-squared: 0.6437

F-statistic: 35.32 on 1 and 18 DF, p-value: 1.267e-05



Example of regression

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set.seed(3)
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Coefficients:

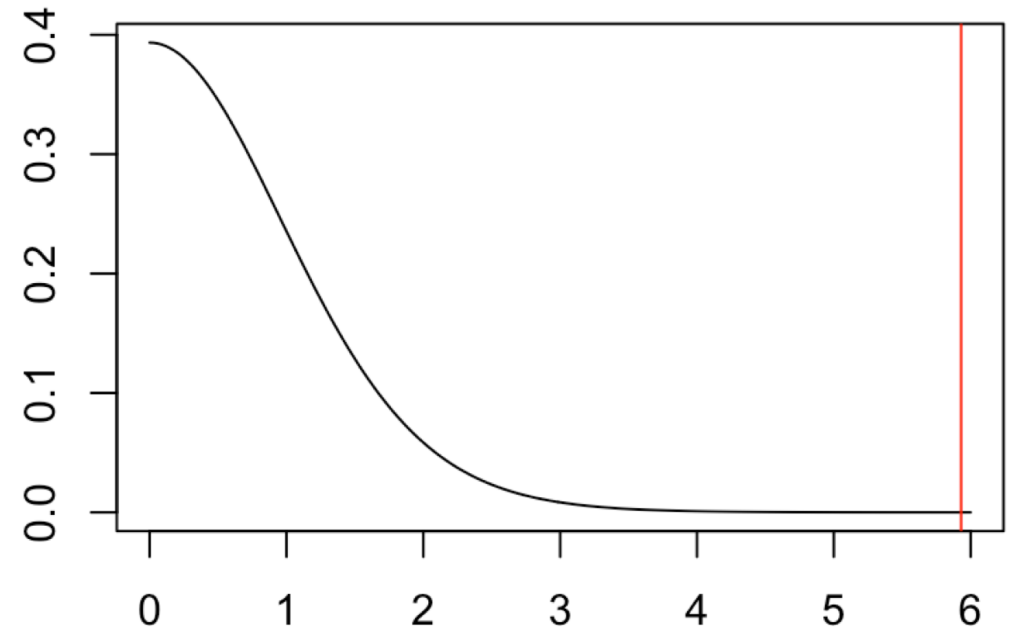
| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|----------|------------|---------|--------------|
| (Intercept) | -0.7173 | 1.0302 | -0.696 | 0.495 |
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t-distribution

Example of regression

```
set.seed(3)
x <- runif(min = 1, max = 10, 20)
y <- rnorm(20, mean = x, sd = 2)
fit.xy <- lm(y ~ x)
summary(fit.xy)
```

Call:
lm(formula = y ~ x)

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|---------|---------|--------|--------|
| -2.7060 | -0.9742 | -0.4539 | 0.9479 | 3.0728 |

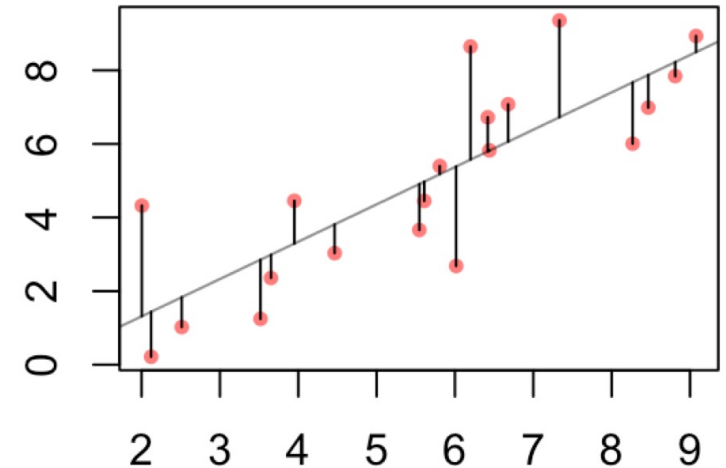
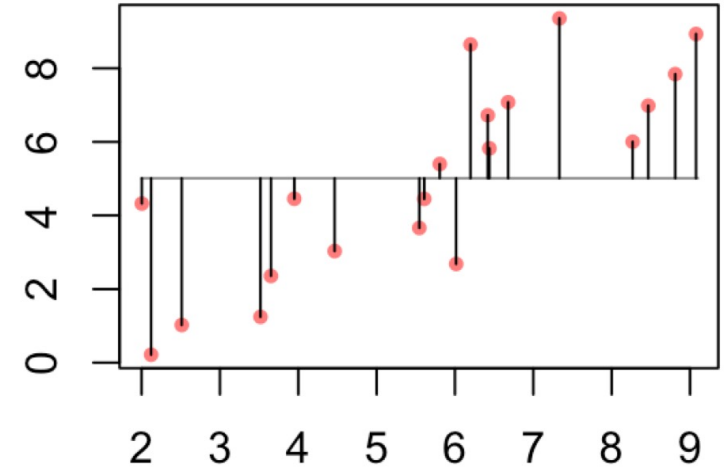
Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|----------|------------|---------|--------------|
| (Intercept) | -0.7173 | 1.0302 | -0.696 | 0.495 |
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Residual standard error: 1.625 on 18 degrees of freedom
Multiple R-squared: 0.6624, Adjusted R-squared: 0.6437
F-statistic: 35.32 on 1 and 18 DF, p-value: 1.267e-05

This can help to justify the biological importance assuming you have a regression that is significant. It is the proportion of total variance explained by the regression.



Multiple vs Adjusted R-squared

Call:

```
lm(formula = y ~ x)
```

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|---------|---------|--------|--------|
| -2.7060 | -0.9742 | -0.4539 | 0.9479 | 3.0728 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|----------|------------|---------|--------------|
| (Intercept) | -0.7173 | 1.0302 | -0.696 | 0.495 |
| x | 1.0150 | 0.1708 | 5.943 | 1.27e-05 *** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.625 on 18 degrees of freedom

Multiple R-squared: 0.6624, Adjusted R-squared: 0.6437

F-statistic: 35.32 on 1 and 18 DF, p-value: 1.267e-05

Adjusted R-squared penalizes for additional parameters

Linear regression uses

- Depict the relationship between two variables in an eye-catching fashion
- Test the null hypothesis of no association between two variables
 - The test is whether or not the slope is zero
- Predict the average value of variable Y for a group of individuals with a given value of variable X
 - variation around the line can make it very difficult to predict a value for a given individual with much confidence
 - Predictions outside of the range of observed data is generally discouraged
- Used both for experimental and observational studies

What are Residuals

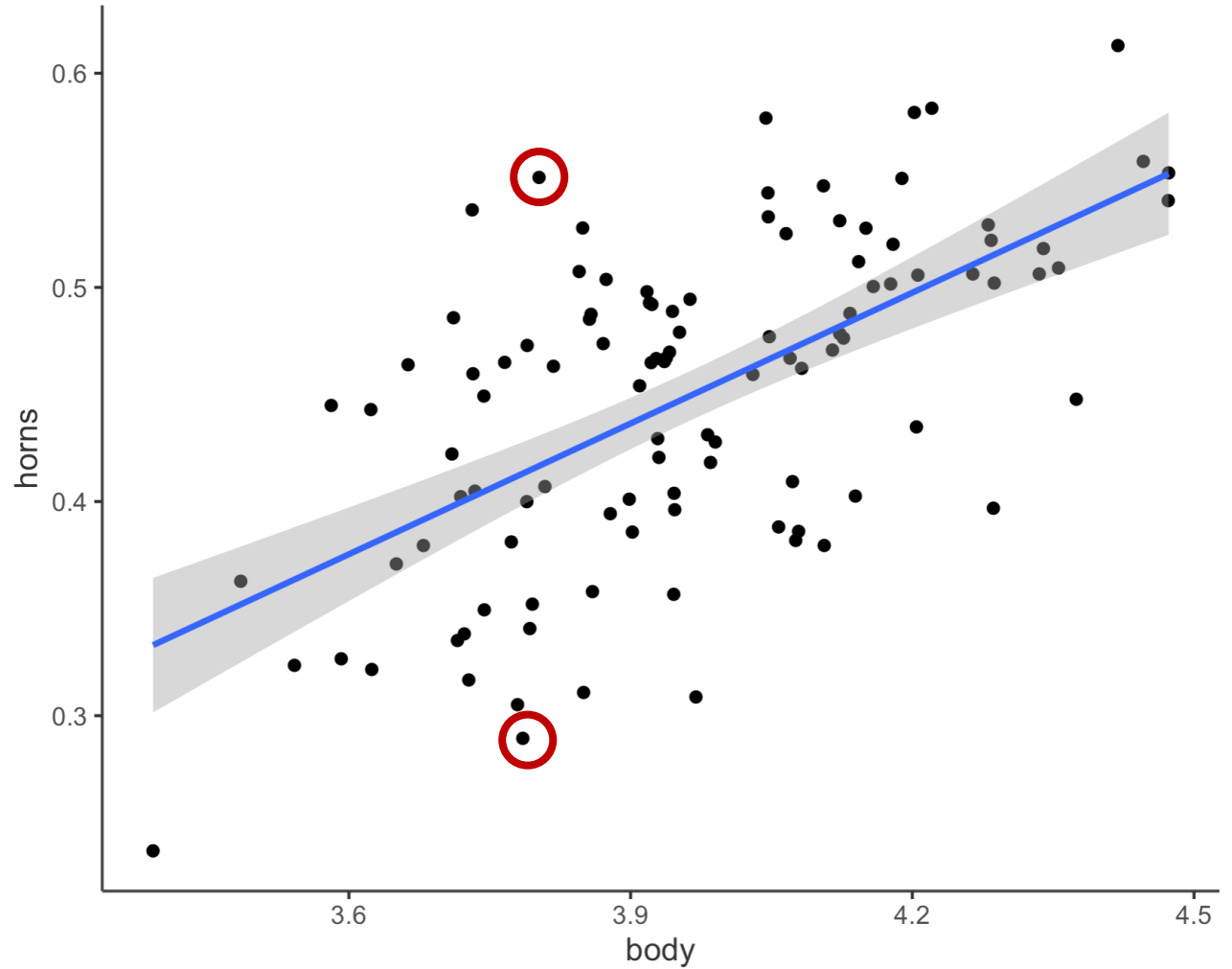
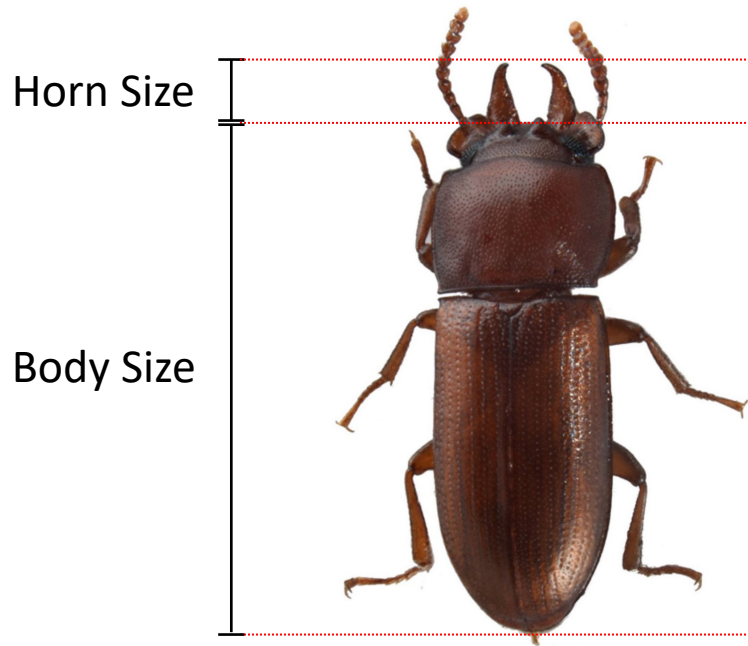
In general, the residual is the individual's departure from the value predicted by the model

In this case the model is simple – the linear regression – but residuals also exist for more complex models

For a model that fits better, the residuals will be smaller on average

Residuals can be of interest in their own right, because they represent values that have been ***corrected*** for relationships that might be obscuring a pattern.

What are Residuals



Making that plot

```
ggtheme <- theme_bw() + theme(panel.grid.major = element_blank(),  
                              panel.grid.minor = element_blank(),  
                              panel.background = element_blank(),  
                              panel.border=element_blank(),  
                              axis.line = element_line(colour="grey30"),  
                              axis.title = element_text(colour="grey20"),  
                              axis.text = (element_text(colour="grey30")),  
                              legend.title = element_text(colour="grey20"),  
                              legend.text = element_text(colour="grey30"))  
  
dat <- read.csv("gnatocerus.csv")  
ggplot(data = dat, aes(x=body, y=horn)) +  
  geom_point() + ggtheme +  
  geom_smooth(method='lm')
```

Strong Inference for Observational Studies

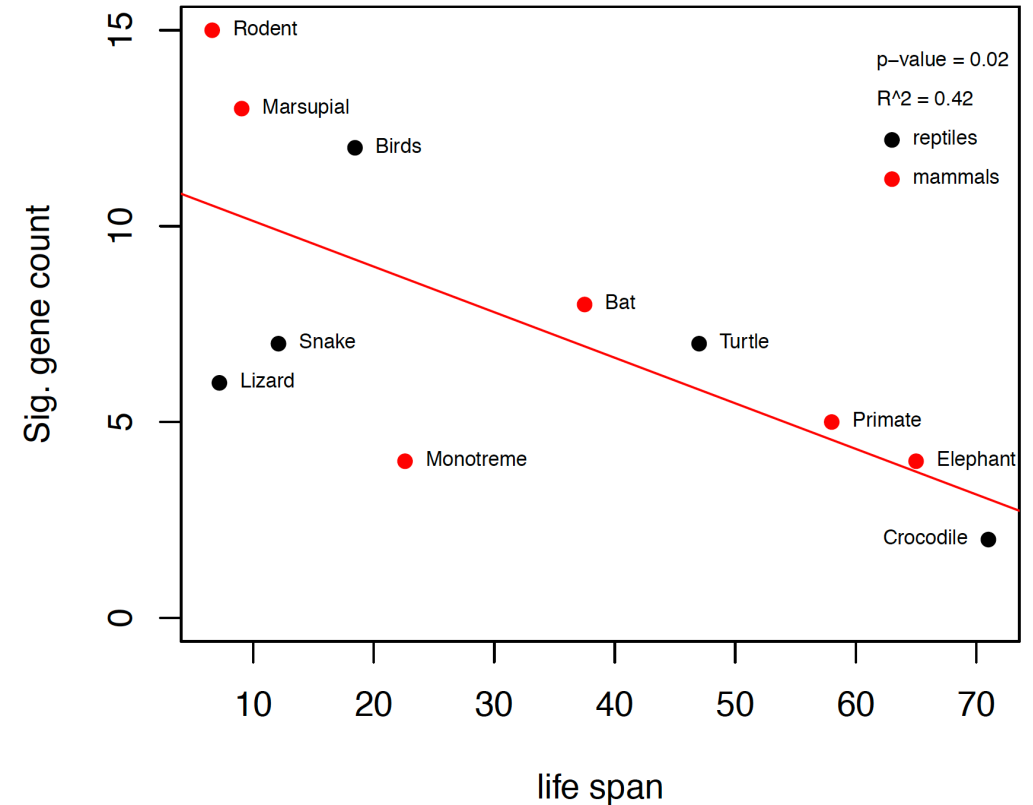
- Noticing a pattern in the data and reporting it represents a post hoc analysis
- This is not hypothesis testing
- The results, while potentially important, must be interpreted cautiously

What can be done?

- Based on a post-hoc observational study, construct a new hypothesis for a novel group or system that has not yet been studied

Example

- 1) We already knew that the P53 network is important in guarding against cancer in long lived species.
- 2) We also knew that primates and elephants show rather little change in this network when compared to rodents.
- 3) Collect data on many more species and test apriori hypothesis that there will be a significant and negative regression coefficient.



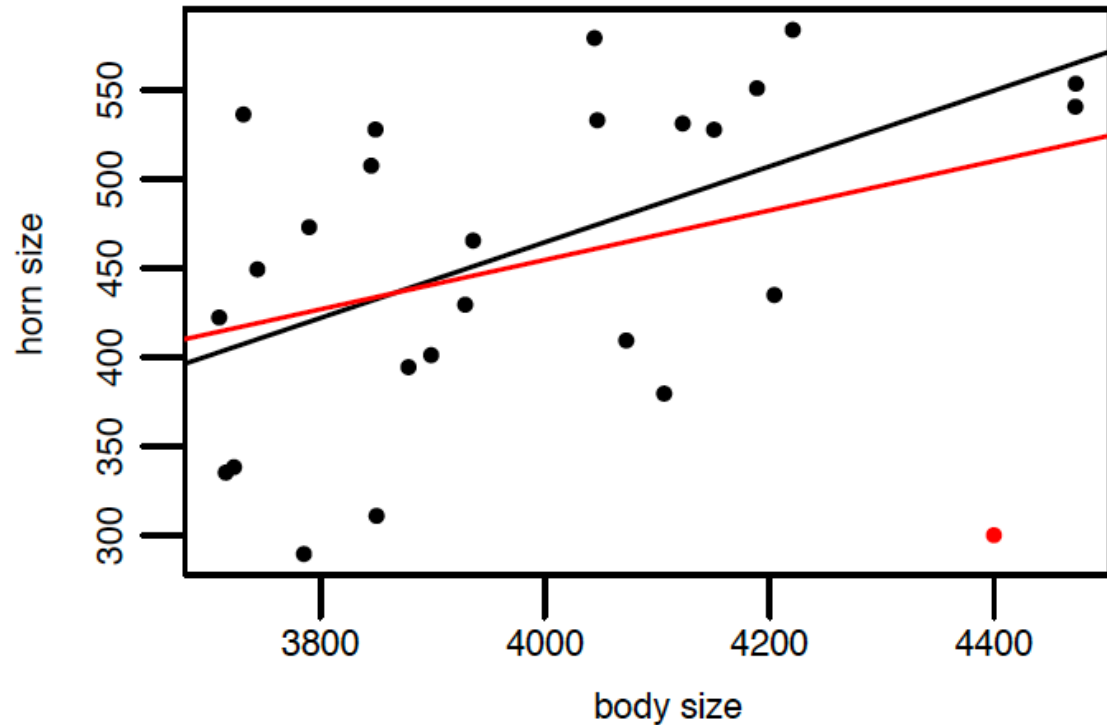
Assumptions of Linear Regression

- The true relationship must be linear
- At each value of X , the distribution of Y is normal (i.e., the residuals are normal)
- The variance in Y is independent of the value of X
- **Note that there are no assumptions about the distribution of X**

Common Problems

- Outliers
 - Regression is extremely sensitive to outliers
 - The line will be drawn to outliers, especially along the x-axis
 - Consider performing the regression with and without outliers
- Non-linearity
 - Best way to notice is by visually inspecting the plot and the line fit
 - Try a transformation to get linearity [often a log transformation]
- Non-normality of residuals
 - Can be detected from a residual plot
 - Possibly solved with a transformation
- Unequal variance
 - Usually visible from a scatterplot or from a residual plot

Outliers



Leverage and cooks distance

Theil–Sen estimator

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|------------|------------|---------|----------|
| (Intercept) | -100.24112 | 297.38717 | -0.337 | 0.7390 |
| x2 | 0.13870 | 0.07431 | 1.867 | 0.0742 . |

Signif. codes:

0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 86.81 on 24 degrees of freedom
Multiple R-squared: 0.1268, Adjusted R-squared: 0.09038
F-statistic: 3.484 on 1 and 24 DF, p-value: 0.07423

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|------------|------------|---------|------------|
| (Intercept) | -386.07048 | 272.48381 | -1.417 | 0.16993 |
| x | 0.21264 | 0.06837 | 3.110 | 0.00493 ** |

Signif. codes:

0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 74.73 on 23 degrees of freedom
Multiple R-squared: 0.296, Adjusted R-squared: 0.2654
F-statistic: 9.673 on 1 and 23 DF, p-value: 0.004928

Homework

- Homework 3
- Use the betta fish data from week 5 and determine whether sex and color have an impact on the price of fish. If color is significant then do a posthoc test to determine which colors are significantly different.
- Use the betta fish data from week 5 and perform a linear regression of price on size. Is the regression significant? What is the Adjusted R-Squared?
- What is the core limitation of the methods that we are using to look at the betta fish data?

Moving past simple models

- The reason ANOVA is so widely used is that it provides a framework to simultaneously test the effects of multiple factors
- ANOVA also makes it possible to detect *interactions* among the factors
- ANOVA is a special case of a *general linear model*
- Linear regression is a special case of a *general linear model*

GLM and LM function in R

- The GLM and LM function in R takes equations that can be described with the following operators
- | | |
|---|--|
| + | +X include this variable |
| : | X:Z include the interaction between these variables |
| * | X*Y include these variables and the interactions between them |
| ^ | (X + Z + W)^3 include these variables and all interactions up to three way |

R versus the math implied

$$\text{glm}(y \sim X + W) \quad y_i = \beta_0 + \beta_1 X_i + \beta_2 W_i + \epsilon_i$$

$$\text{glm}(y \sim X * W) \quad y_i = \beta_0 + \beta_1 X_i + \beta_2 W_i + \beta_3 X_i W_i + \epsilon_i$$

R versus the math oak example

Call:
glm(formula = specialist ~ temp * circ, data = oak)

Deviance Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|---------|---------|--------|--------|
| -4.2804 | -1.1295 | -0.2256 | 0.9952 | 5.6787 |

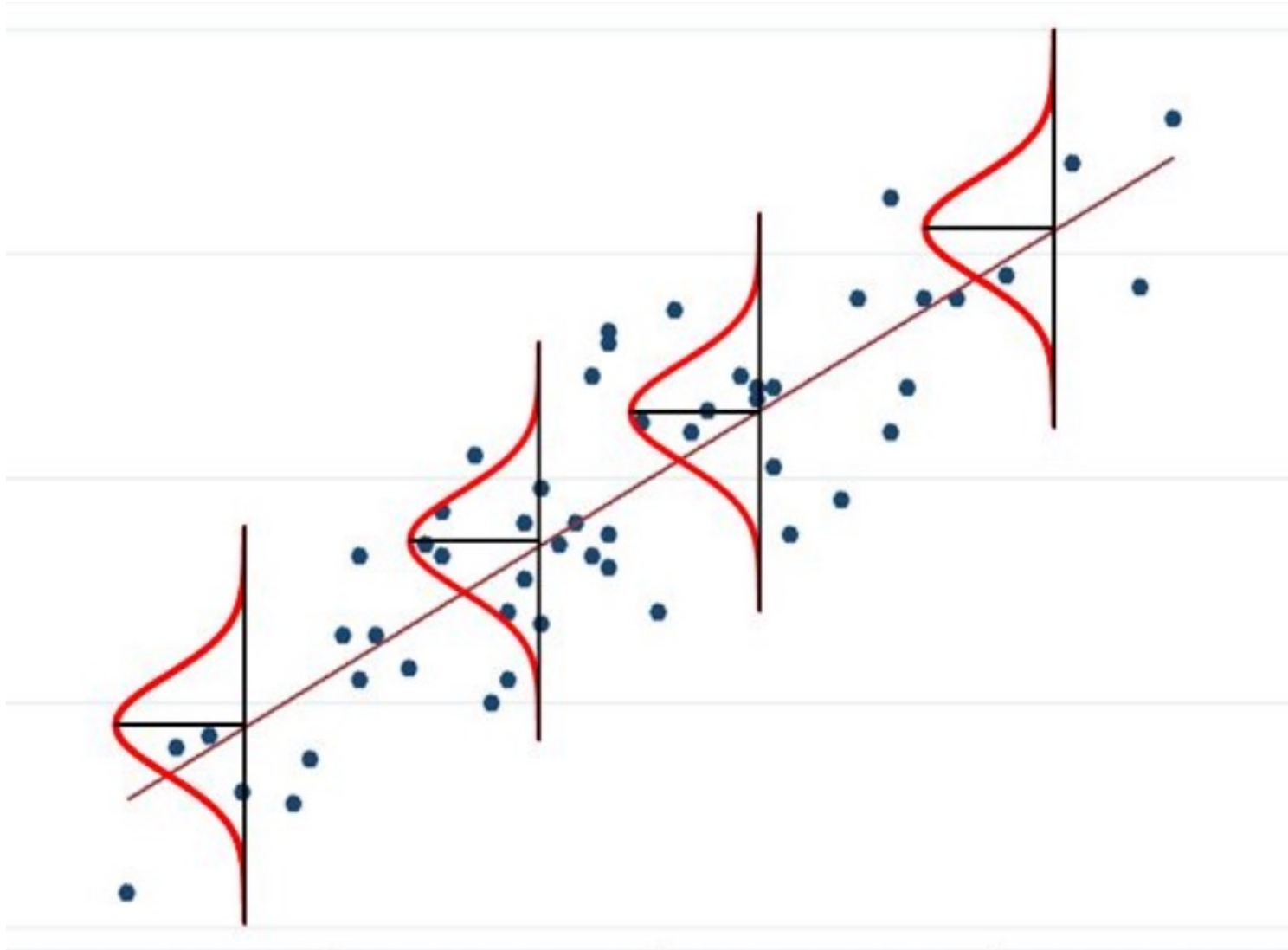
Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|-------------|------------|------------|---------|----------|
| (Intercept) | 9.7621149 | 3.8327598 | 2.547 | 0.0114 |
| temp | -0.5574479 | 0.2527323 | -2.206 | 0.0282 |
| circ | -0.0661544 | 0.0120692 | -5.481 | 9.40e-08 |
| temp:circ | 0.0045895 | 0.0007887 | 5.819 | 1.61e-08 |

| circ | temp | precip | specialist |
|-------|------|--------|------------|
| 592.0 | 15.8 | 257 | 3 |
| 680.0 | 14.7 | 455 | 1 |
| 340.0 | 14.5 | 458 | 1 |
| 310.0 | 14.5 | 458 | 4 |
| 260.0 | 14.5 | 458 | 2 |

$$y_i = \beta_0 + \beta_1 temp_i + \beta_2 circ_i + \beta_3 temp_i circ_i$$

When the response variable isn't normal



Other kinds of regression

Logistic regression allows us to fit a binary response variable (absent/present; alive/dead) with one or more categorical or continuous predictor variables.

Poisson regression allows us to fit a response variable that is Poisson distributed (number of extinctions in a unit of time, number of colonies per plate, (number of occurrences for rare events)) with one or more categorical or continuous predictor variables.

```
fit.logi <- glm(obs ~ pred2 , family="binomial")
```

```
fit.pois <- glm(obs ~ pred2, family="poisson")
```