ANOVAs and Generalized Linear Models

ANOVAs T-tests Binomial Test Chi-Square Test

CURVE-FITTING METHODS AND THE MESSAGES THEY SEND QUADRATIC LOGARITHMIC . LINEAR "HEY, I DIDA "I WANTED A CURVED "LOOK, IT'S REGRESSION." LINE, SO I MADE ONE TAPERING OFF!" UITH MATH." EXPONENTIAL LOESS LINEAR NO SLOPE "LOOK. IT'S GROWING "I'M SOPHISTICATED, NOT "I'M MAKING A LIKE THOSE BUMBLING UNCONTROLLABLY!" SCATTER PLOT BUT POLYNOMIAL PEOPLE." I DON'T WANT TO." LOGISTIC CONFIDENCE PIECEWISE INTERVAL "I NEED TO CONNECT THESE "LISTEN, SCIENCE IS HARD. "I HAVE A THEORY, TWO LINES, BUT MY FIRST IDEA BUT I'M A SERIOUS AND THIS IS THE ONLY DIDN'T HAVE ENOUGH MATH." PERSON DOING MY BEST." DATA I COULD FIND. CONNECTING AD-HOC HOUSE OF I INFS FILTER CARD5 "I CLICKED 'SMOOTH "I HAD AN IDEA FOR HOW "AS YOU CAN SEE, THIS LINES' IN EXCEL." TO CLEAN UP THE DATA. MODEL SMOOTHLY FITS THE- WAIT NO NO DON'T WHAT DO YOU THINK?" EXTEND IT AAAAAA!!"

	Common name	Built-in function in R	Equivalent linear model in R	Exact?	The linear model in words	lcon
(x +	y is independent of x P: One-sample t-test N: Wilcoxon signed-rank	t.test(y) wilcox.test(y)	lm(y ~ 1) lm(signed_rank(y) ~ 1)	✓ for N >14	One number (intercept, i.e., the mean) predicts y . - (Same, but it predicts the <i>signed rank</i> of y .)	- 1
: Im(y ~ 1	P: Paired-sample t-test N: Wilcoxon matched pairs	t.test(y ₁ , y ₂ , paired=TRUE) wilcox.test(y ₁ , y ₂ , paired=TRUE)	lm(y₂ - y₁ ~ 1) lm(signed_rank(y₂ - y₁) ~ 1)	✓ f <u>or N >14</u>	One intercept predicts the pairwise y ₂ - y ₁ differences. - (Same, but it predicts the <i>signed rank</i> of y ₂ - y ₁ .)	
regression	y ~ continuous x P: Pearson correlation N: Spearman correlation	cor.test(x, y, method='Pearson') cor.test(x, y, method='Spearman')	$lm(y \sim 1 + x)$ lm(rank(y) ~ 1 + rank(x))	√ <u>for N >10</u>	One intercept plus x multiplied by a number (slope) predicts y . - (Same, but with <i>ranked</i> x and y)	- Alexandre
Simple r	y ~ discrete x P: Two-sample t-test P: Welch's t-test N: Mann-Whitney U	t.test(y ₁ , y ₂ , var.equal=TRUE) t.test(y ₁ , y ₂ , var.equal=FALSE) wilcox.test(y ₁ , y ₂)	$Im(y \sim 1 + G_2)^A$ $gls(y \sim 1 + G_2, weights=^B)^A$ $Im(signed_rank(y) \sim 1 + G_2)^A$	✓ ✓ 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 .)	¥.
X ₂ +)	P: One-way ANOVA N: Kruskal-Wallis	aov(y ~ group) kruskal.test(y ~ group)	$Im(y \sim 1 + G_2 + G_3 + + G_N)^A$ Im(rank(y) ~ 1 + G ₂ + G ₃ + + G _N) ^A	✓ for N >11	An intercept for group 1 (plus a difference if group ≠ 1) predicts y . - (Same, but it predicts the <i>rank</i> of y .)	÷ <u></u> ţ‡ŧ
- 1 + X ₁ +	P: One-way ANCOVA	aov(y ~ group + x)	$Im(y \sim 1 + G_2 + G_3 + + G_N + x)^4$	~	- (Same, but plus a slope on x.) Note: this is discrete AND continuous. ANCOVAs are ANOVAs with a continuous x.	
ssion: Im(y ~	P: Two-way ANOVA	aov(y ~ group * sex)	$Im(y \sim 1 + G_2 + G_3 + + G_N + S_2 + S_3 + + S_K + G_2^*S_2 + G_3^*S_3 + + G_N^*S_K)$	*	Interaction term: changing sex changes the y ~ group parameters. Note: $G_{2 \text{ to } N}$ is an <u>indicator (0 or 1)</u> 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 _i) 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 .	[Coming]
ultiple regression:	Counts ~ discrete x N: Chi-square test	chisq.test(groupXsex_table)	$\begin{array}{c} \mbox{Equivalent log-linear model} \\ glm(y \sim 1 + G_2 + G_3 + + G_N + \\ S_2 + S_3 + + S_K + \\ G_2 ^* S_2 + G_3 ^* S_3 + + G_N ^* S_K , \mbox{ family=})^A \end{array}$	~	Interaction term: (Same as Two-way ANOVA.) Note: Run glm using the following arguments: glm (model, family=poisson()) As linear-model, the Chi-square test is $log(y_i) = log(N) + log(\alpha_i) + log(\alpha_i\beta_j) + log(\alpha_i\beta_j)$ where α_i and β_j are proportions. See more info in <u>the accompanying notebook</u> .	Same as Two-way ANOVA
Mu	N: Goodness of fit	chisq.test(y)	$glm(y \sim 1 + G_2 + G_3 + + G_N, family=)^A$	~	(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)

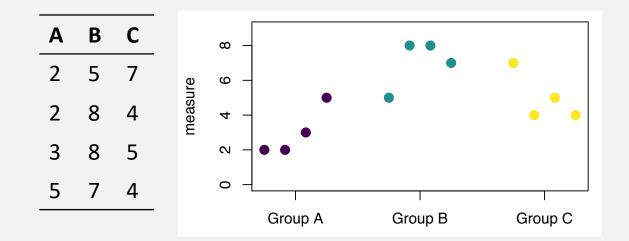
ON THE "PROBABLE ERROR" OF A COEFFICIENT OF CORRELATION DEDUCED FROM A SMALL SAMPLE

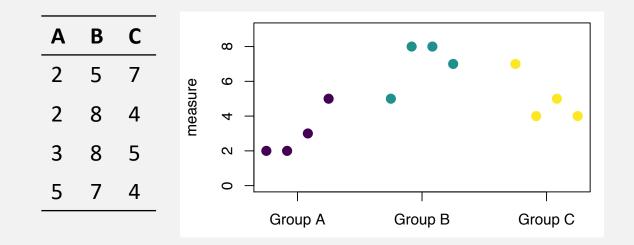
Fisher 1921

Author's Note (CMS 1.2a)

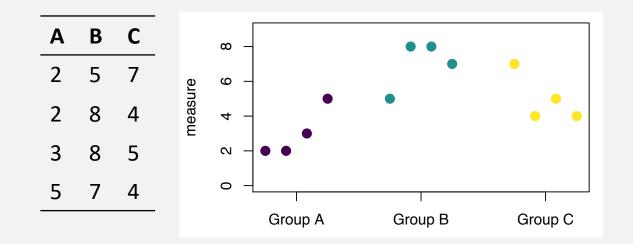
This is the second of three papers dealing with the sampling errors of correlation coefficients covering the cases (i) "The frequency distribution of the values of the correlation coefficient in samples from an indefinitely large population," *Biometrika*, Vol. 10, pp. 507-521, 1915.

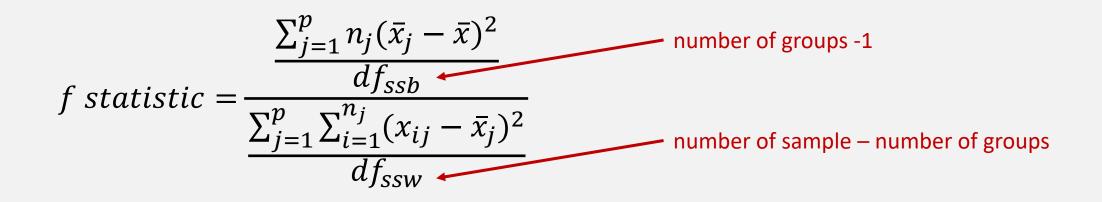
- Used to compare the means among more than two groups
- If you are comparing three groups, for instance, you cannot just do three pair-wise *t*-tests – this approach would cause too many false positives
- ANOVA takes into account the fact that you are comparing multiple groups and controls the false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

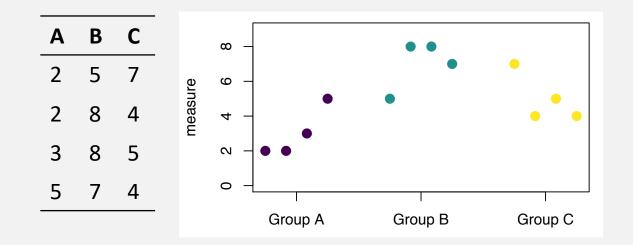




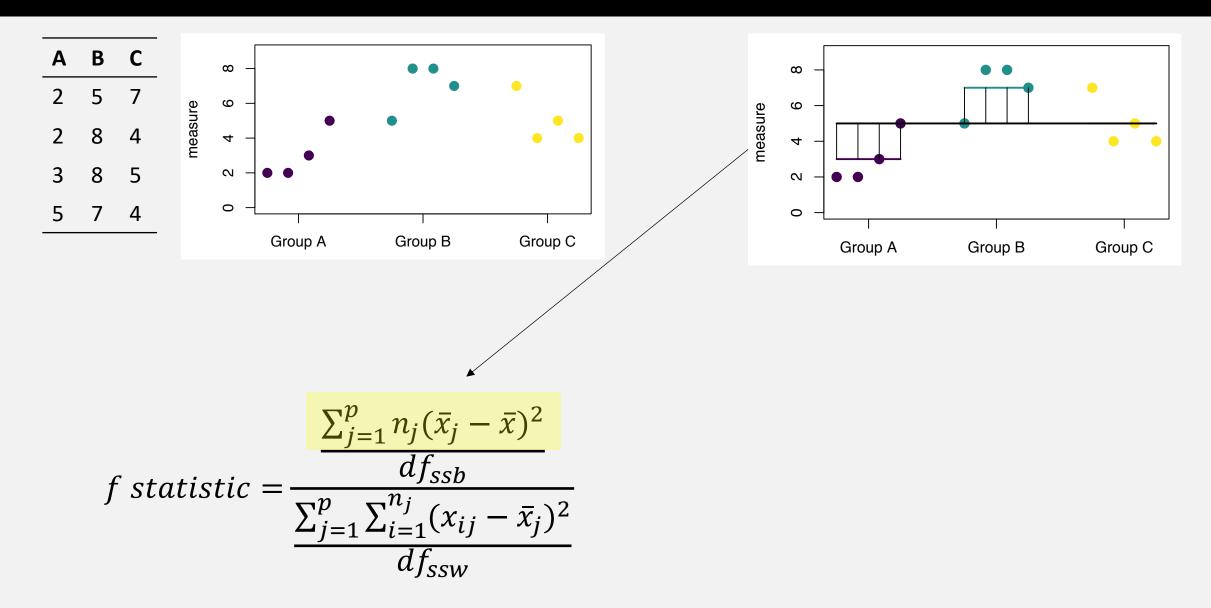
$$f \ statistic = \frac{\frac{\sum_{j=1}^{p} n_j (\bar{x}_j - \bar{x})^2}{df_{ssb}}}{\frac{\sum_{j=1}^{p} \sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{df_{ssw}}}$$

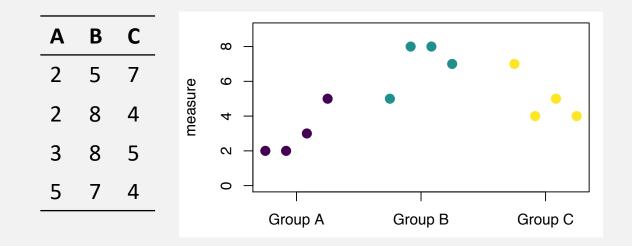


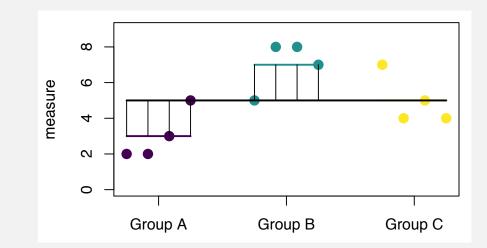




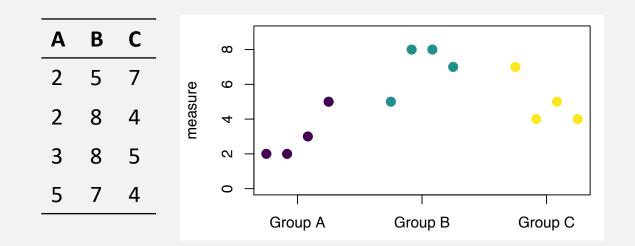
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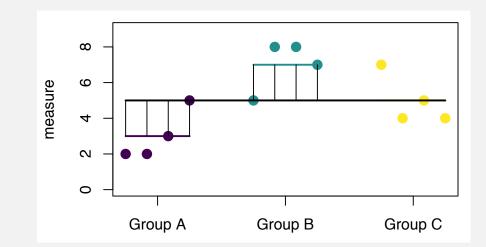


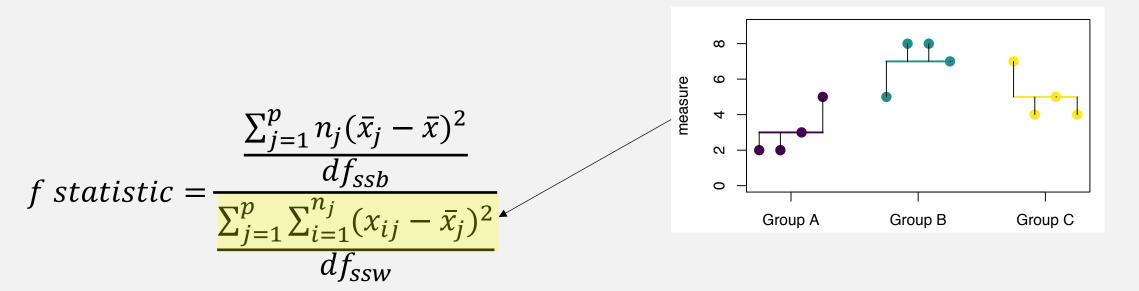




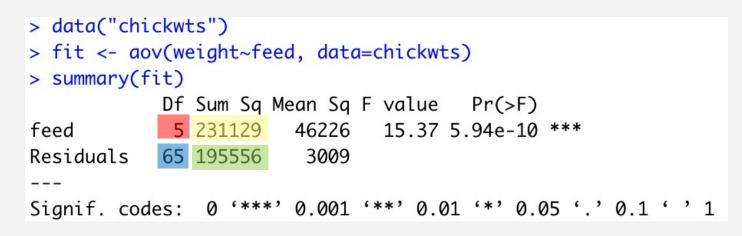
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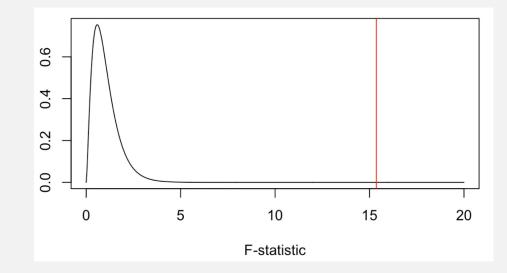






Running ANOVA in R

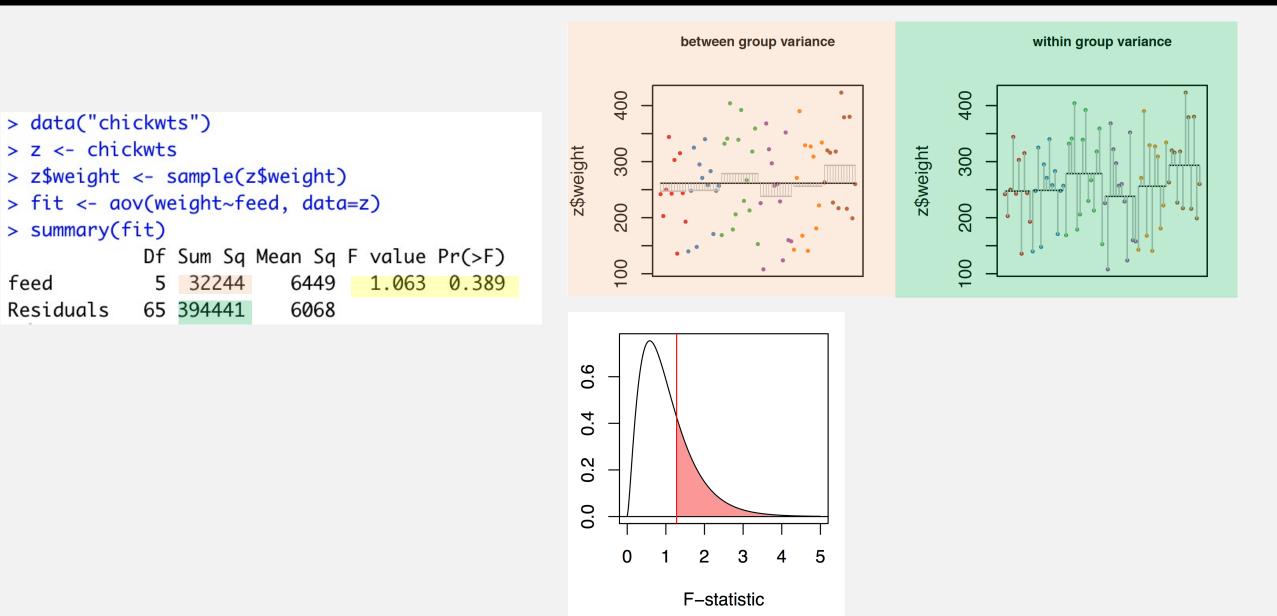




This significant result tells us that at least one of the groups of chickens have significantly different mean weights than at least one other groups. (significant ANOVA result allows us to reject the null that they are all the same)

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

Running ANOVA in R



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)</pre>

> 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)</pre>

```
> TukeyHSD(fit)
```

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

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

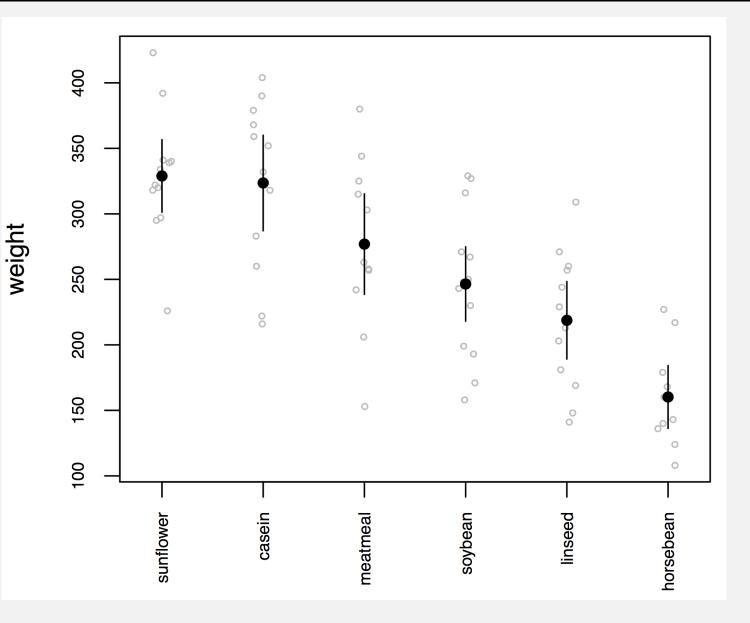
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When we examine all the significantly different ones we can draw several conclusions:

- Chicks fed casein are significantly heavier than those fed horsebean, linseed, and soybean.
- Chicks fed horsebean are significantly lighter than those fed meatmeal, soybean, and sunflower.
- 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,</pre>
                         levels=c("sunflower", "casein",
                                   "meatmeal", "soybean",
                                   "linseed", "horsebean"))
stripchart(weight ~ feed, data=chickwts,
           method = "jitter", vertical = TRUE, cex.axis =.7,
           col = "qray", pch = 1, cex = .5, las = 3
#Add error bars:
#First calculate means and SDs
meanShift <- tapply(chickwts$weight, chickwts$feed, mean)</pre>
sdevShift <- tapply(chickwts$weight, chickwts$feed, sd)</pre>
n <- tapply(chickwts$weight, chickwts$feed, length)</pre>
feed_table <- data.frame(mean = meanShift,</pre>
                          std.dev = sdevShift, n = n
#Now add the SEM for each group:
seShift <- 1.96 * sdevShift / sqrt(n)</pre>
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 |
                        horsebea
               casein
                                    linseed
                                              meatmeal
                                                          soybean
horsebea l
             4.813069
              0.0000*
             3.308292 -1.658736
 linseed
              0.0141*
                          1.0000
             1.415755 -3.364059
meatmeal
                                  -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.
- Use the offspring.csv file from the course website and determine whether XY and ZW systems have different numbers of male offspring.

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

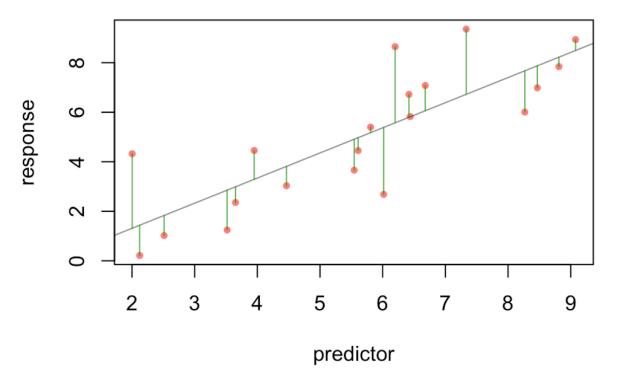
- 1. Linear regression
- 2. Poisson regression
- 3. Binomial regression
- 4. GLMs with a mix of variable types
- 5. Mixed effects models

Regression in R

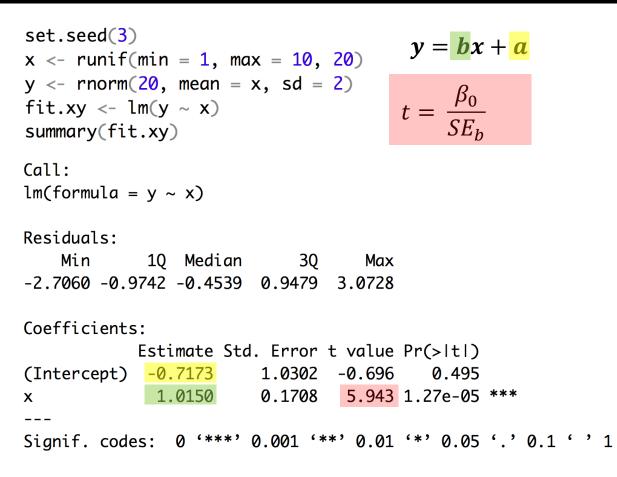
 With linear regression we find the linear equation that best predicts the values of Y based on the values of X.

$$2) y = bx + a$$

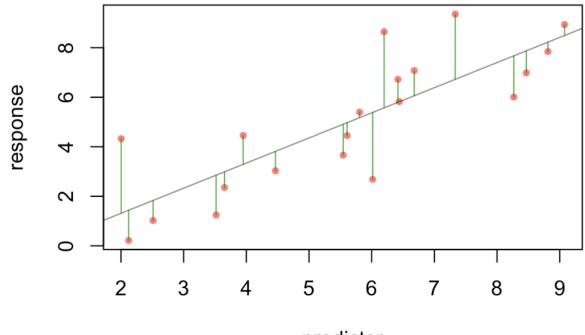
 Least-squares regression minimizes the squared deviations of the data points from that line.



Example of regression

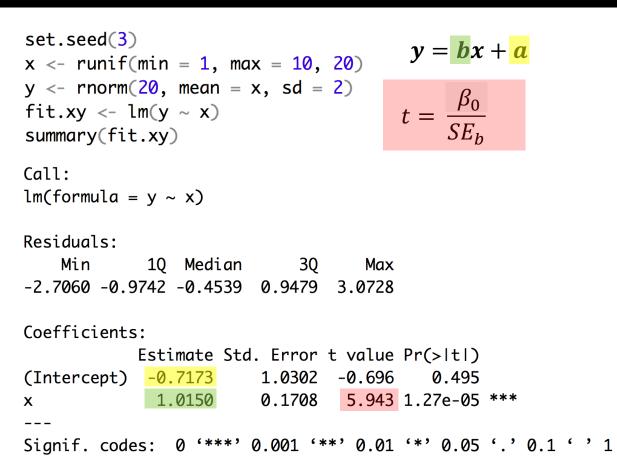


Residual standard error: 1.625 on 18 degrees of freedom Multiple R-squared: 0.6624, Adjusted R-squared: 0.6437 F-statistic: 35.32 on 1 and 18 DF, p-value: 1.267e-05

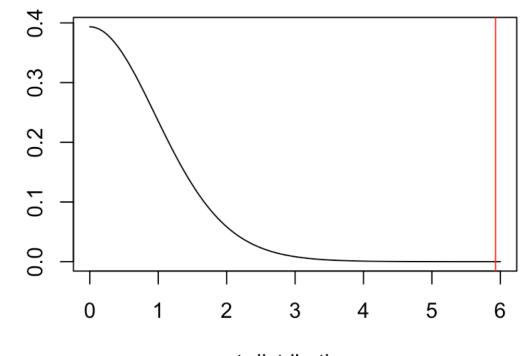


predictor

Example of regression

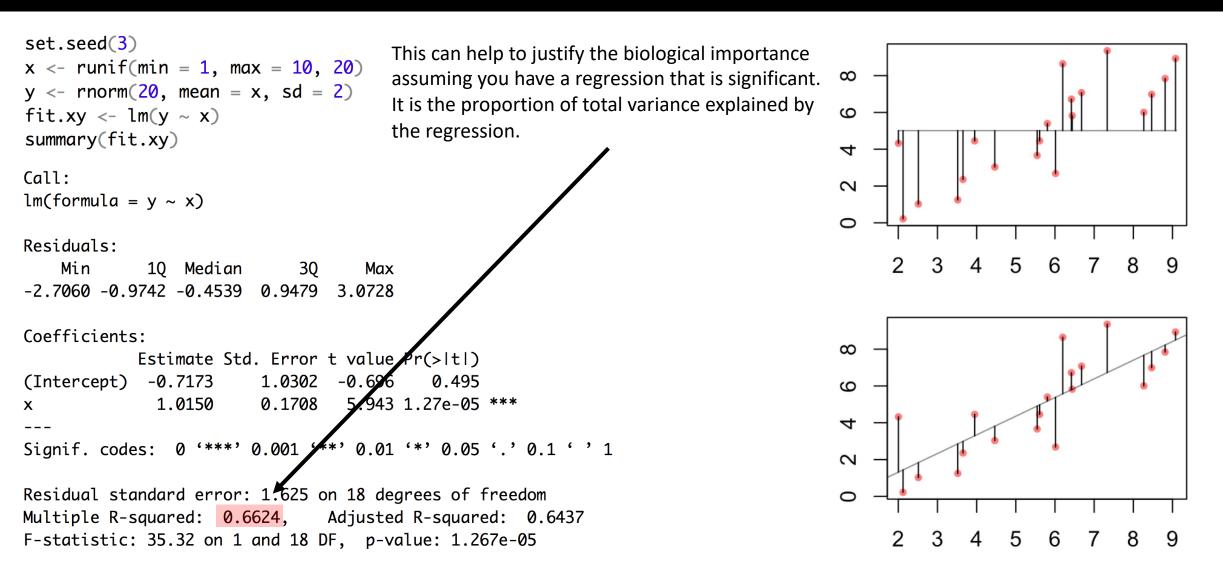


Residual standard error: 1.625 on 18 degrees of freedom Multiple R-squared: 0.6624, Adjusted R-squared: 0.6437 F-statistic: 35.32 on 1 and 18 DF, p-value: 1.267e-05



t-distribution

Example of 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

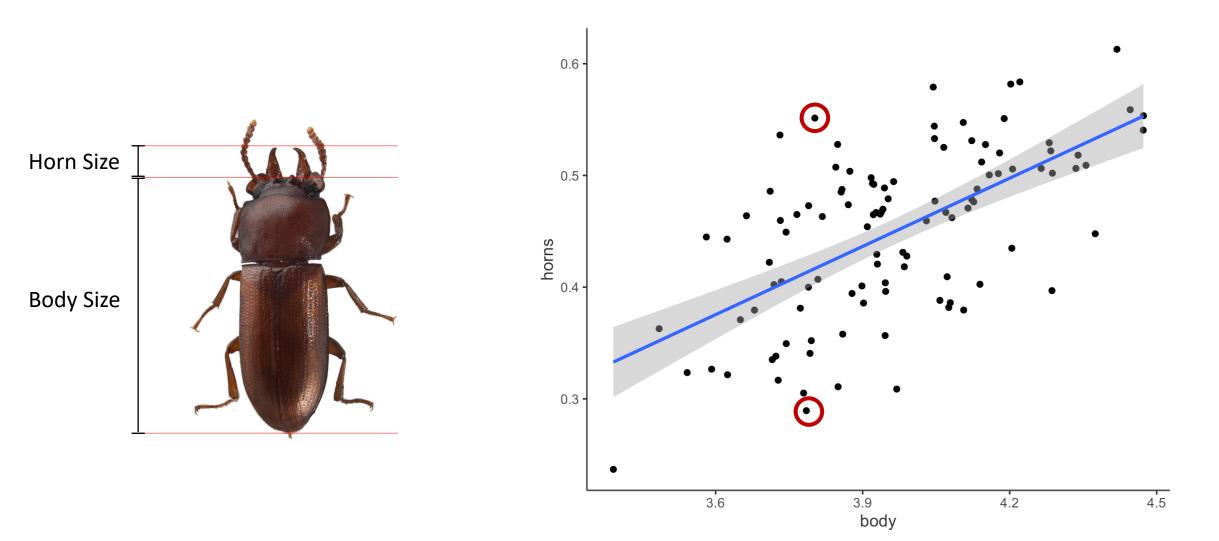
In general, the residual is the individual's departure from the value predicted by the model

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")</pre>
ggplot(data = dat, aes(x=body, y=horns)) +
  geom_point() + ggtheme +
  geom_smooth(method='lm')
```

Strong Inference for Observational Studies

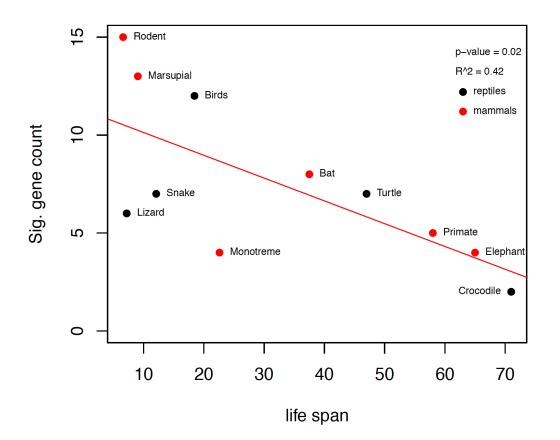
- Noticing a pattern in the data and reporting it represents a post hoc analysis
- This is not hypothesis testing
- The results, while potentially important, must be interpreted cautiously

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

- 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.
- Collect data on many more species and test apriori hypothesis that there will be a significant and negative regression coefficient.



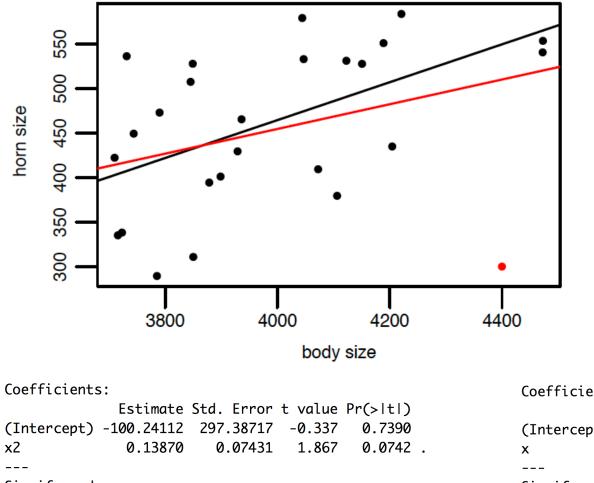
Assumptions of Linear Regression

- 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

(Intercept) -100.24112 297.38717 -0.337 0.7390 x2 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 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

$$glm(y \sim X + W) \qquad y_i = \beta_0 + \beta_1 X_i + \beta_2 W_i + \epsilon_i$$

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

R versus the math oak example

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

Deviance Residuals:

Min	1Q	Median	3Q	Мах
-4.2804	-1.1295	-0.2256	0.9952	5.6787

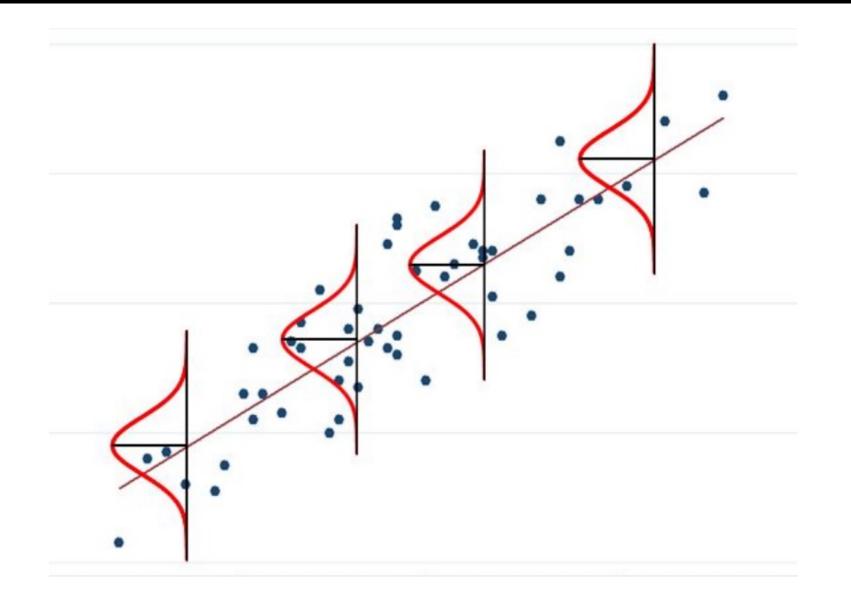
Coefficients:

	Estimate	Std. Error	t value	Pr(>ltl)
(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")</pre>

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