Regression Models & ANCOVA

Advanced Biostatistics

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Lecture 4

EEOB 590C

Regression: Y~X

-X & Y are continuous

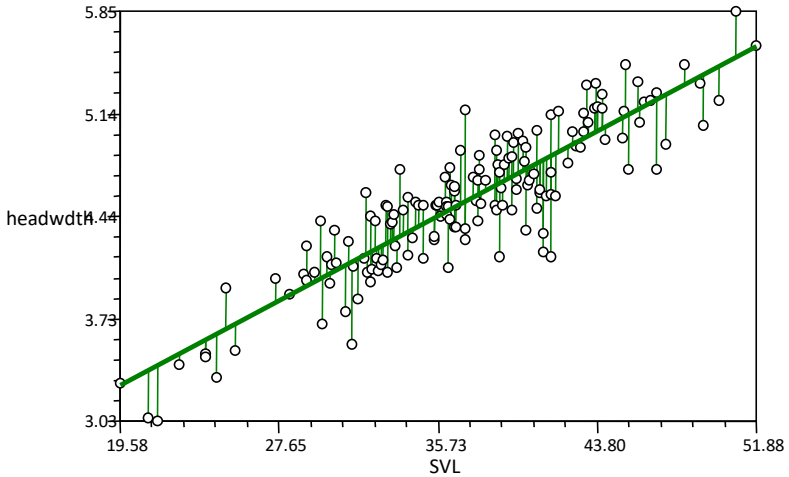
H_0: no relationship between X & Y

-Compare variation explained by model to residual error variation

Model: $Y_i = \beta_0 + \beta_i X_i + \varepsilon_i$ (β is grand mean, β_1 is slope, and ε_{ij} is error)

-Standard test statistic: F-ratio (ratio of variances)

$$F \approx \frac{\sigma^2_{model}}{\sigma^2_{error}}$$



Name from Galton's work: offspring values 'regress' (tend towards) to that of parents

1: Independence: ε_{ij} of variates must be independent

2: Normality: requires normally distributed ε_{ij}

3: Homoscedasticity: equal variance

-Samples along regression line are homoscedastic (variance doesn't 'balloon' along regression)

As increase x value, y variance increases dramatically. (Ice cream cone)

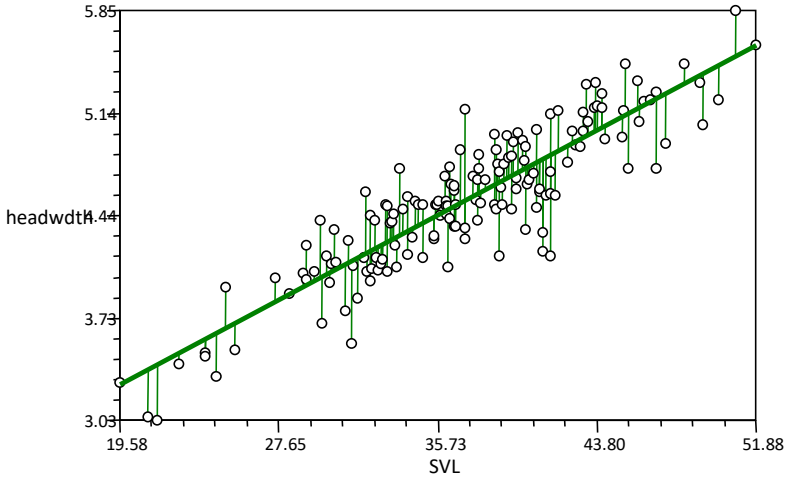
4: X values are independent and measures *without error*

Fit line that minimizes sum of squared deviations (LS fit) from Y-variates to line (vertical deviations b/c no error in X)

How much does Y "wiggle and squiggle" with X relative to the variation in X?

Slope calculated as: $b_{Y \cdot X} = \dfrac{\sum_{i=1}^{n}\left(X_i - \bar{X}\right)\left(Y_i - \bar{Y}\right)}{\sum_{i=1}^{n}\left(X_i - \bar{X}\right)^2}$ $\left(\sum(cross\ products) \Big/ SS_x \right)$

Regression line always crosses $\left(\bar{X}, \bar{Y}\right)$ so intercept is: $\beta_0 = \bar{Y} - b_{Y \cdot X}\bar{X}$



Note: this is Model I regression with 1 Y for each X. Slight alterations exist for multiple Y for each X: see Biometry.

Can obtain SS_{Model} & SS_{Error}

Y ~ X

SS explained: $SSR = SSM = \sum_{i=1}^{n}\left(\hat{Y}_i - \bar{Y}\right)^2$ where $\hat{Y}$ are predicted values

SS error: $SSE = \sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2$

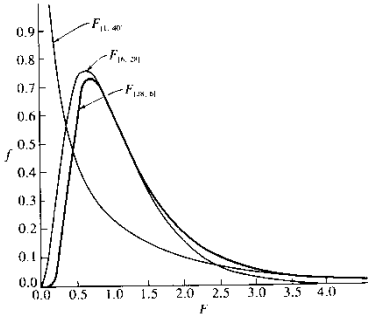
Traditional test: F-ratio (MSR / MSE)

Source	df	SS	MS	F	P
Regression	1	SSM	SSM/df	MSM/MSE	
Error	n-2	SSE	SSE/df		
Total	n-1	SST=SSM+SSE			

Tests whether regression explains a significant portion of variation

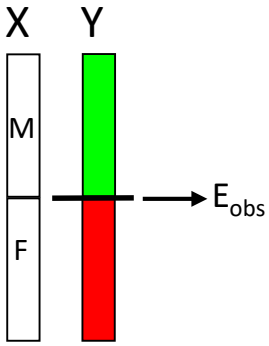
Standard approach:

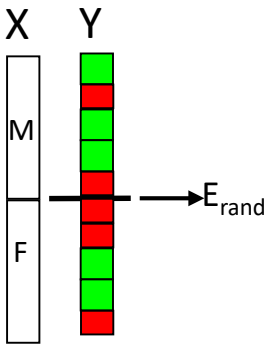
-Compare F-ratio to F-distribution with appropriate df

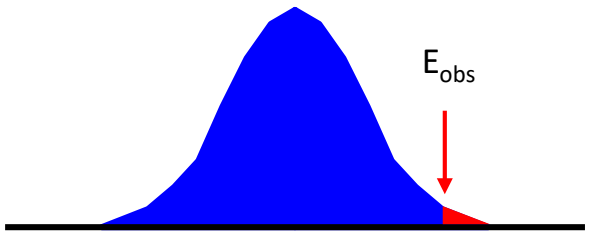


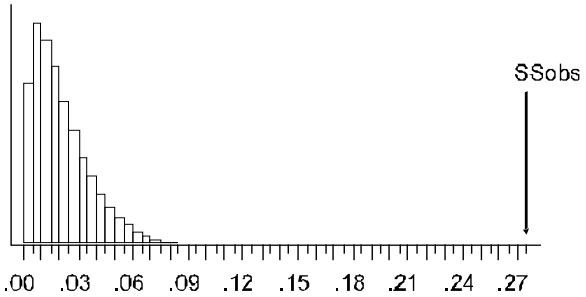
Resampling Alternative:

-Shuffle **Y** relative to **X** & generate distribution of possible outcomes









F-test assesses variance explained, but not how much Y changes with X

Can evaluate the model parameters separately

Slope test ($\beta_1 \neq 0$): $t = \beta_1 - 0 \,/\, s_{\beta_1}$ with $s_{\beta_1} = \sqrt{\frac{MSE}{\sum_{i=1}^{n}(X_i - \bar{X})^2}}$ (n-2 df)

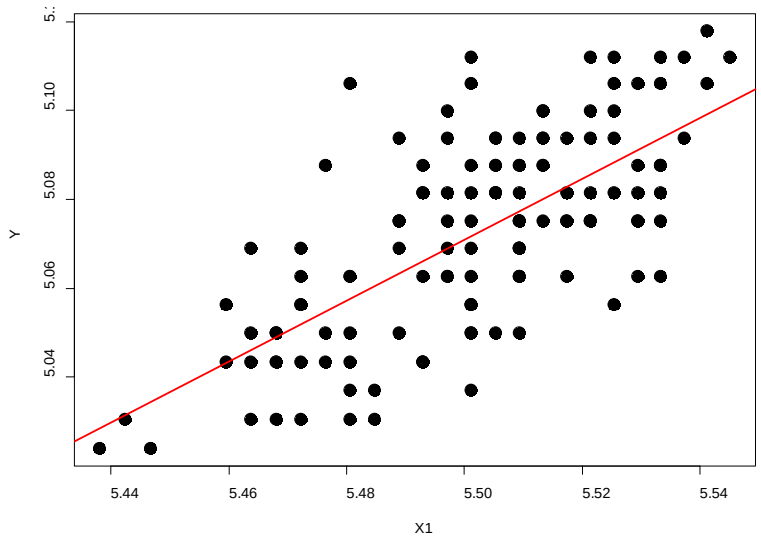
Intercept test ($\beta_0 \neq 0$): $t = \beta_0 - 0 \,/\, s_{\beta_0}$ with $s_{\beta_0} = \sqrt{MSE\left[\frac{1}{n} + \frac{\bar{X}^2}{\sum_{i=1}^{n}(X_i - \bar{X})^2}\right]}$ (n-2 df)

Can also test against particular values (e.g,. Isometry: is $\beta_1 = 1$?)

Very useful for certain biological hypotheses

```
> summary(lm(Y~X1))#TotalLength ~ AlarExtent
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   1.3045     0.3383   3.856 0.000179 ***
X1            0.6848     0.0615  11.136  < 2e-16 ***
Multiple R-squared: 0.4806,    Adjusted R-squared: 0.4768
```

```
> anova(lm(Y~X1))#provides regression coefficients
           Df   Sum Sq  Mean Sq F value    Pr(>F)
X1          1 0.032412 0.032412  124.01 < 2.2e-16 ***
```



Y= 1.3045 + 0.6848X

Correlation and regression closely linked

Mathematically: $b_{Y \cdot X} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{SS_x}$ vs. $r_{xy} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{s_x s_y}$

Sums of squares of X: standard deviation of X times standard deviation of X

Difference in denominator, so related as: $r_{xy} = b_{Y \cdot X}\left(s_x / s_y\right)$

r is a *standardized* regression coefficient (i.e. slope in standard deviation units)

-β: error in Y direction only (direction of scatter)

-*r*: error in X & Y (dispersion of scatter)

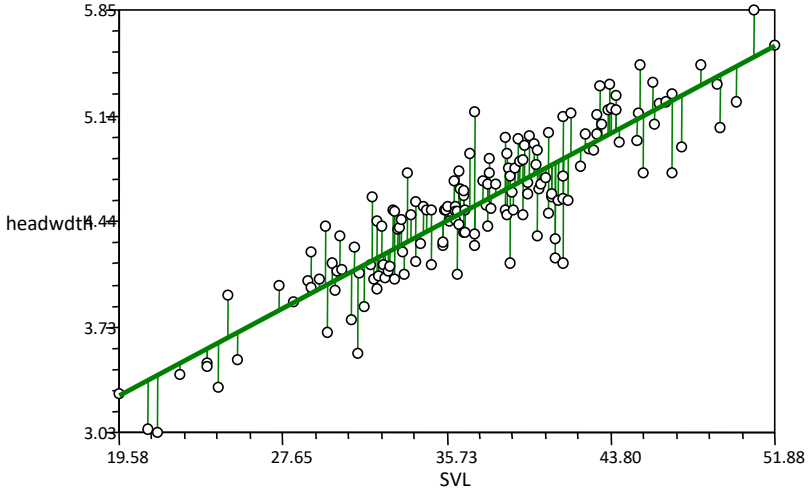
-Thus, regression models *causation* (Y as function of X) and correlation models association (covariation in X & Y)

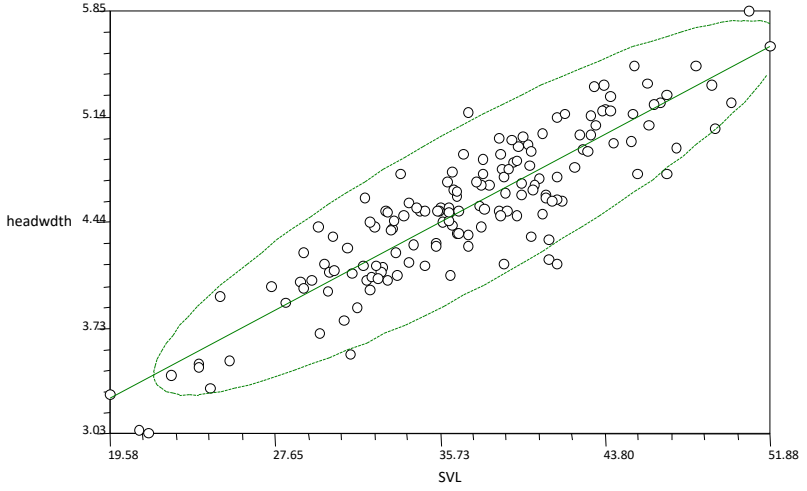
Regression

$$b_{Y \cdot X} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{SS_x}$$

Correlation

$$r_{xy} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{s_x s_y}$$





When both X & Y contain measurement error, model I regression underestimates slope

-Model II regression accounts for this by minimizing deviations perpendicular to regression line (not in Y direction only)

-Different types of model II regression, depending on data (major axis, reduced major axis, etc.)

-X & Y in ‘same’ units/scale: major axis regression (PCA)

-X & Y not in same units/scale: standard (reduced) major axis regression

Principle Components Analysis

When X & Y in 'same' units/scale: major axis regression (PCA)

Compute covariance matrix for X & Y:

$$VCV = \begin{bmatrix} s_X^2 & s_{XY} \\ s_{XY} & s_Y^2 \end{bmatrix}$$

Square and symmetric

Obtain principle eigenvalue (λ) and eigenvector

$$\lambda_1 = \frac{s_X^2 + s_Y^2 + \sqrt{\left(s_X^2 + s_Y^2\right)^2 - 4\left(s_X^2 s_Y^2 - s_{XY}^2\right)}}{2}$$

This equation to find length of first skewer in data cloud.

Slope is: $$b_{Y \cdot X} = \frac{s_{XY}}{\lambda_1 - s_X^2}$$

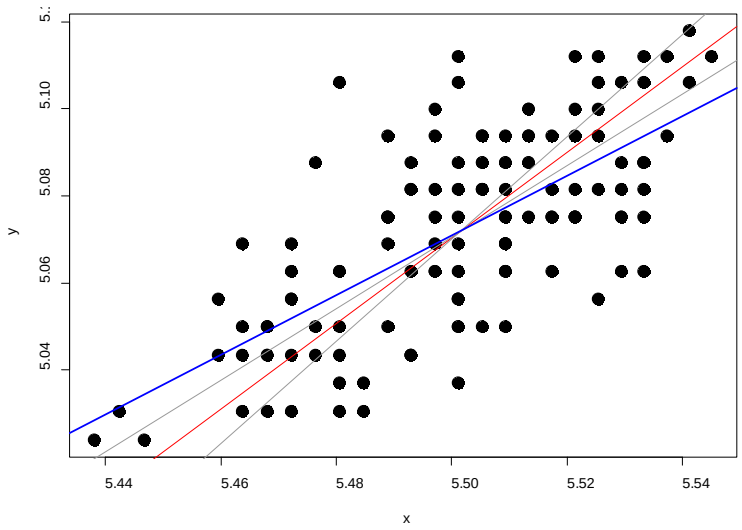
When X & Y not in same units/scale: standard (reduced) major axis regression

Standardize variables to $N(0,1)$ (standard normal deviates)

Calculate SMA slope as: $$b_{Y \cdot Xrma} = \frac{b_{Y \cdot X}}{r_{XY}}$$

```
> lmodel2(Y~X1,nperm=999)
Angle between the two OLS regression lines = 20.53316 degrees

Method  Intercept     Slope  Angle (degrees)  P-perm (1-tailed)
1    OLS  1.3044593 0.6847984         34.40328              0.001
2     MA -0.3329159 0.9824064         44.49152              0.001
3    SMA -0.3624212 0.9877692         44.64746                 NA
```



OLS slope (blue)

MA slope (red)

*SMA slope = β_{OLS}/r

***NOTE: Justification for RMA usage has been overplayed in biology.**

-What matters is *NOT* whether X has measurement error: var(ε_x)

-Instead, *ONLY* when var(ε_x)/var(X) is large, might there be an issue with Model I regression

-But even in this case, RMA is not guaranteed to alleviate the problem

(see Kilmer & Rodriguez, 2017. *J. Evol. Biol.*)

Identify relationship between several X and continuous Y

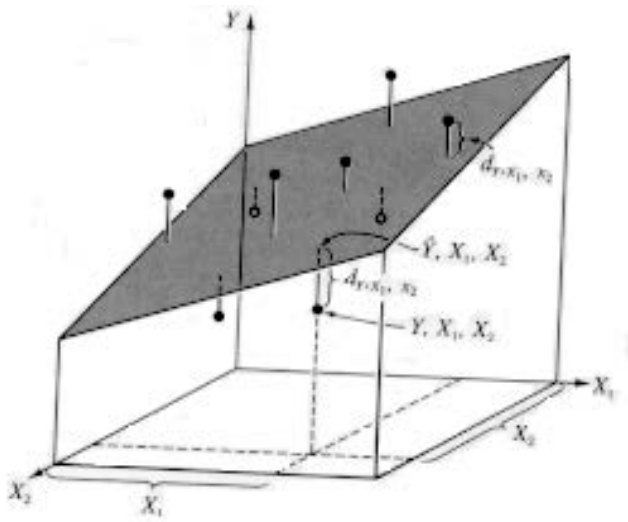
Predict Y using several variables simultaneously

Y ~ A + B

Model: $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \varepsilon_i$

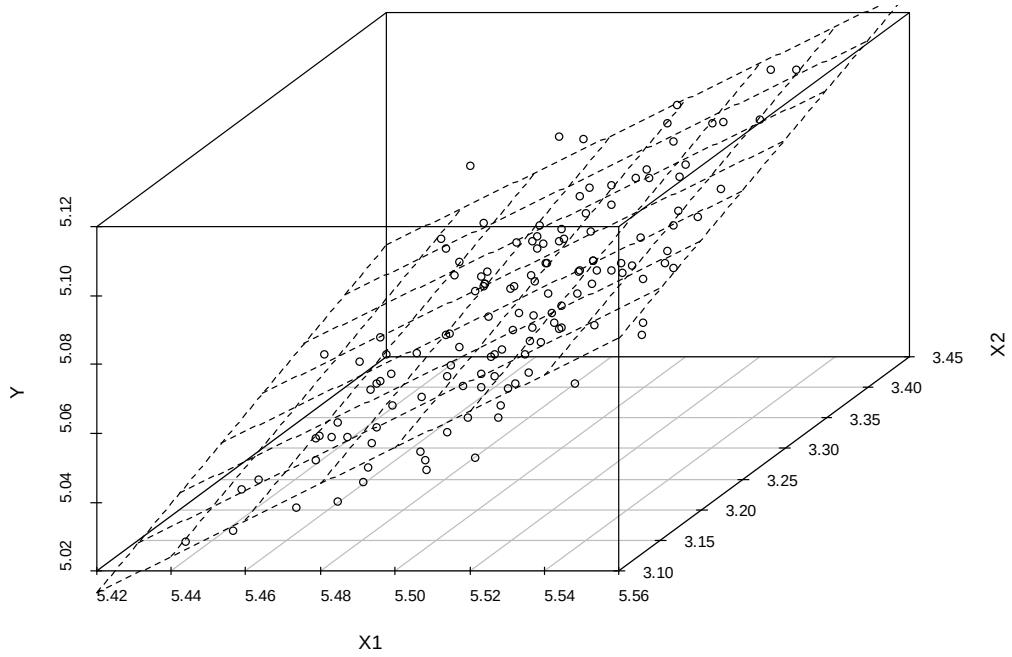
β_i are partial regression coefficients (effect of X_i while holding effects of other X constant)

For 2X, think of fitting a plane to the data



```
> summary(lm(Y~X1+X2))
Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.82521    0.34843   5.238 6.18e-07 ***
X1           0.52516    0.07142   7.354 1.77e-11 ***
X2           0.11042    0.02835   3.895 0.000155 ***

> anova(lm(Y~X1+X2))
Df   Sum Sq  Mean Sq F value    Pr(>F)
X1          1 0.032412 0.032412 137.118 < 2.2e-16 ***
X2          1 0.003585 0.003585  15.168 0.0001551 ***
```



Standard partial regression coefficients $b'_{YX_i \cdot X_j}$: β of Y & X_i holding X_j constant.

Expresses change in normalized units (standard normal deviates: $\frac{Y - \overline{Y}}{\sigma_Y}$)

ADVANTAGE: can be directly compared for each variable

Found from variable correlations: $b'_{YX_1 \cdot X_2} = \frac{r_{X_1 Y} - r_{X_2 Y} r_{X_1 X_2}}{1 - r^2_{X_1 X_2}}$

Interpret, then calculate back to original units and for conventional partial regression coefficients

$$b_{YX_1 \cdot X_2} = b'_{YX_1 \cdot X_2} \frac{S_Y}{S_{X1}}$$

For all models, R^2: proportion of variance explained by model

Should I add another variable? Test difference in R^2

NOTE: R^2 will 'top' out as you add variables (so frequently adjusted R^2 used)

Can also compare models using 'aov' in R, using AIC, etc.

How to add variables: stepwise addition, stepwise deletion, random addition, etc.

NOTE: different methods yield different results (b/c $b'_{YX_i \cdot X_j}$ depend on other variables in model)

CAREFUL WITH BIOLOGICAL INTERPRETATION!!!

To compare 2 regression lines, calculate F-ratio as:

$$F = \frac{\left(b_1 - b_2\right)^2}{\frac{\sum\left(X_1 - \bar{X}_1\right)^2 + \sum\left(X_2 - \bar{X}_2\right)^2}{\left(\sum\left(X_1 - \bar{X}_1\right)^2\right)\left(\sum\left(X_2 - \bar{X}_2\right)^2\right)} \bar{s}^2_{Y \bullet X}}$$

Df = 1, $(n_1 + n_2 - 4)$

Where $\bar{s}^2_{Y \bullet X}$ is the weighted average of $s^2_{Y \bullet X}$

Procedure can be generalized to compare > 2 regression lines (see *Biometry*)

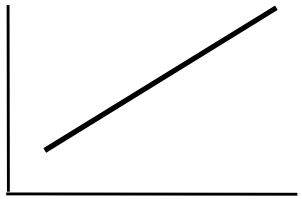
-Often, one wishes to compare regression lines AND the groups

-ANCOVA 'combines' regression and ANOVA

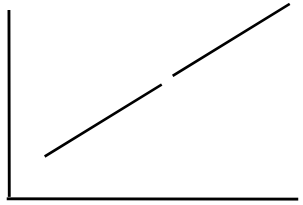
-H_0: no difference among slopes, no difference among groups

-Must first compare slopes, then compare groups (ANOVA) while holding effects of covariate constant

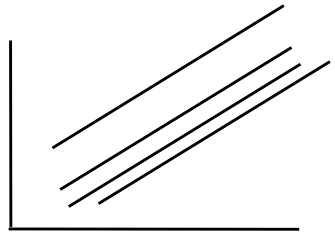
-Several possible outcomes



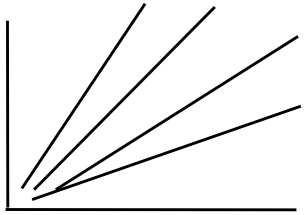
Groups the same, slopes the same (overlapping data)



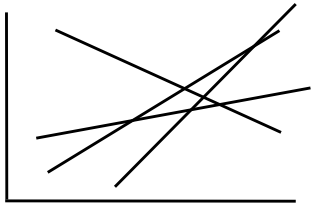
Groups the same, slopes the same (non -overlapping data)



Slopes the same, groups different



Slopes different, 'easily' identified pattern



Slopes different, 'complicated' pattern

Model: $$Y_{ij} = \mu + \alpha_i + \beta_{within}\left(X_{ij} - \overline{X}_i\right) + \varepsilon_{ij}$$

Calculate SS for:

1: covariate (common regression)

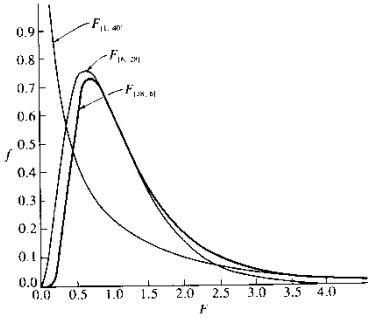
2: group x covariate interaction (slopes test)

3: groups

Source	df	SS	MS	F	P
Covariate	1	SSR	SSR/df		
Group	a-1	SSG	SSG/df		
Cov x Group	a-1	SSInt	SSInt/df		
Error	n-2(a-1)+1	SSE	SSE/df		

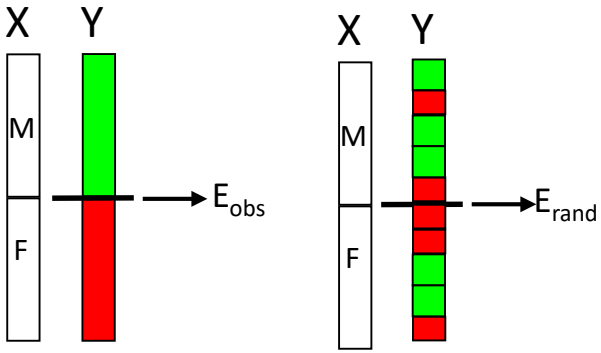
Standard approach:

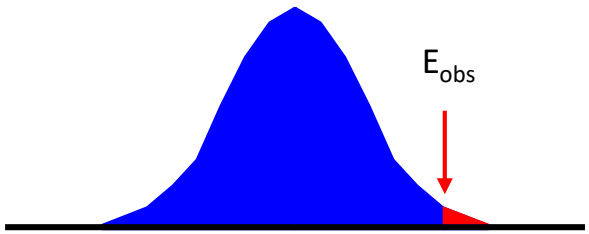
-Compare F-ratio for each factor to F-distribution with appropriate df

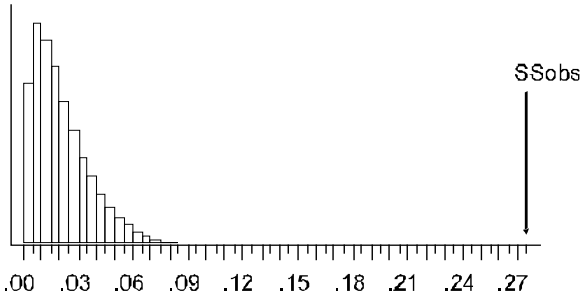


Resampling Alternative:

-Residual randomization: shuffle residuals from reduced model to assess that factor (e.g., remove A×cov and use randomization to test A×cov factor)







1: Are slopes for groups different ($SS_{covXgroup} > 0$?)

2: If interaction significant, then *we cannot compare groups,* because slopes differ

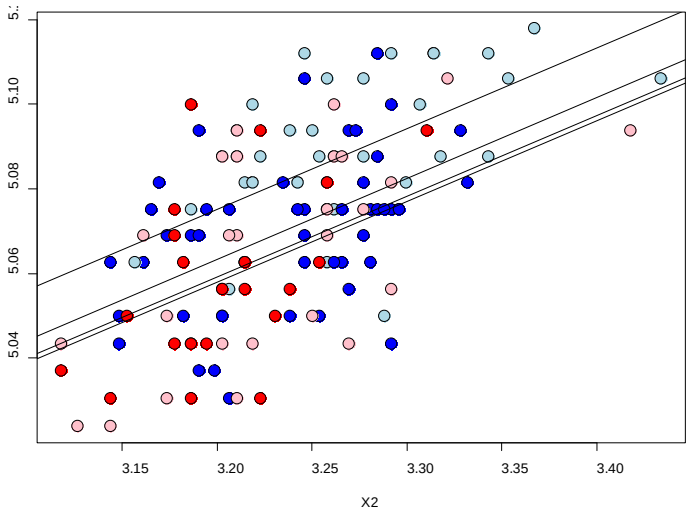
Group comparisons not useful, as the variation is in their group-specific slopes

3: If interaction term is NOT significant, then test groups while holding covariate constant (ie, refit new model as Y~cov + group)

```
> anova(lm(Y~X2*SexBySurv))
               Df   Sum Sq   Mean Sq F value    Pr(>F)
X2              1 0.023215 0.0232149 77.3890 8.139e-15 ***
SexBySurv       3 0.005172 0.0017239  5.7467  0.001014 **
X2:SexBySurv    3 0.000651 0.0002172  0.7239  0.539496
Residuals     128 0.038397 0.0003000
```

Fit common-slope model

```
> anova(lm(Y~X2+SexBySurv))
            Df   Sum Sq   Mean Sq F value    Pr(>F)
X2           1 0.023215 0.0232149 77.8814 6.015e-15 ***
SexBySurv    3 0.005172 0.0017239  5.7833 0.0009591 ***
```



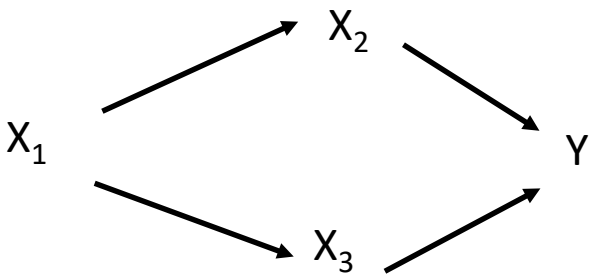
1: Perform ANOVA on regression residuals: NOT the same as ANCOVA (different df, pooled β, etc.). Also, lose test of slopes, which is important (see J. Anim. Ecol. 2001. 70:708-711)

2: Significant cov:gp interaction, but still compare groups: not useful, as answer depends upon where along regression you compare

3: "Size may be a covariate, so I'll use a small size range to 'standardize' for it": choosing animals of similar sizes will eliminate covariate, but also will eliminate potentially important biological information (e.g., what if male head width grows relatively faster than females (i.e. size:head interaction?)

Many other models possible

-PATH ANALYSIS & SEM (structural equation modeling): looks at partial correlation coefficients and partial regression coefficients to explain variance in data according to hypothesized 'causal' path between variables



Curvilinear regression: Fit polynomials of X: X, X^2, X^3, etc.

Regression an incredibly flexible tool for testing hypotheses

Can add factors, based on theory of partitioning SS

Careful in decisions here!

Permutations also incredibly useful for hypothesis testing