# Analysis of Variance (ANOVA)

Sumanta Basu

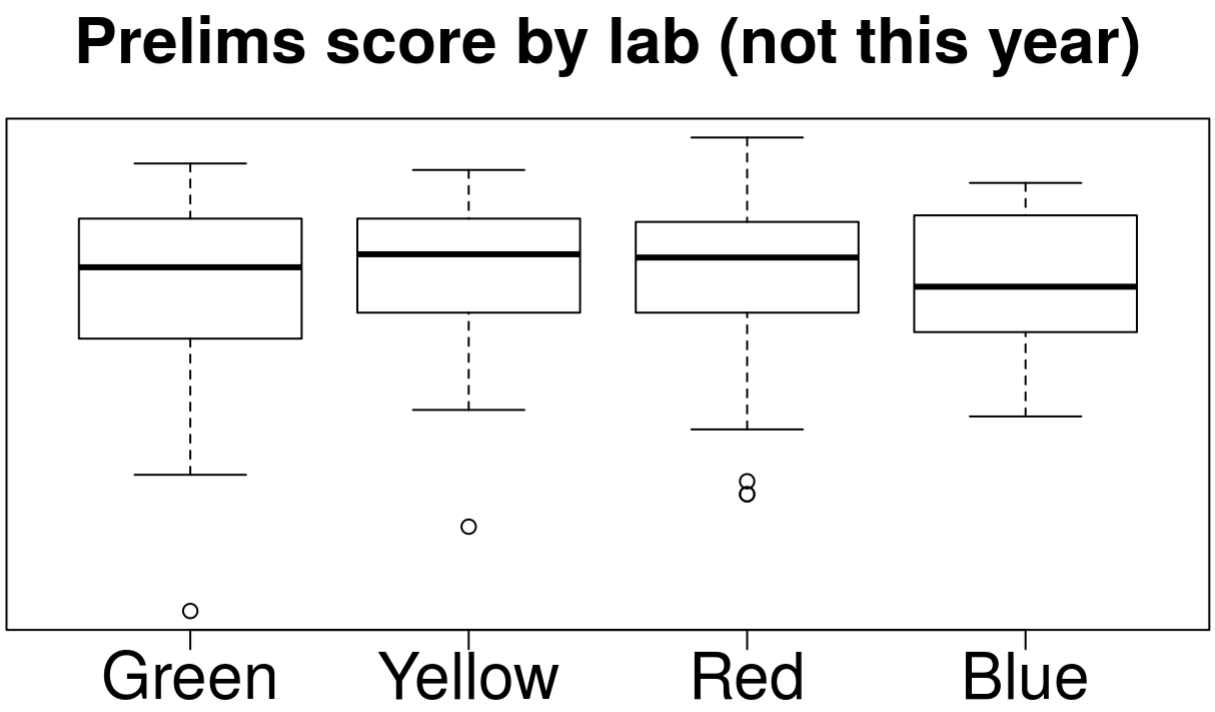
# Comparing more than two means with ANOVA

# Outline 1

> *How about we compare all possible pairs?*

- *Pro: Can simply use t-tests many times*
- *Con: Type-I error inflates quickly*
- *Correct for multiple hypothesis testing, at the expense of losing power*

# Is there a difference between labs?



# Should we just compare all pairs?

There are $\binom{4}{2} = 6$ pairs: Could do a two-sample test for each:

- $H_0 : \mu_1 = \mu_2$
- $H_0 : \mu_1 = \mu_3$
- $H_0 : \mu_1 = \mu_4$
- $H_0 : \mu_2 = \mu_3$
- $H_0 : \mu_2 = \mu_4$
- $H_0 : \mu_3 = \mu_4$

Not a good idea.

# Multiple testing problem

Suppose we do $M$ independent tests at significance level $\alpha$. Suppose all nulls are true.

$$P(\text{Fail to reject all } H_0 \mid \text{all } H_0\text{'s are true}) = (1-\alpha)^M$$

Suppose we write up a paper on our findings if we rejected any of the nulls. **What's the probability of writing a paper when we shouldn't?**

$$P(\text{Reject at least 1 } H_0 \mid \text{all } H_0\text{'s are true}) = 1-(1-\alpha)^M$$

- E.g. if $\alpha = 0.05$ and $M = 6$, this is $26\%$.
- if $M = 13$, it's about $50\%$
- if $M = 30$, it's nearly $80\%$

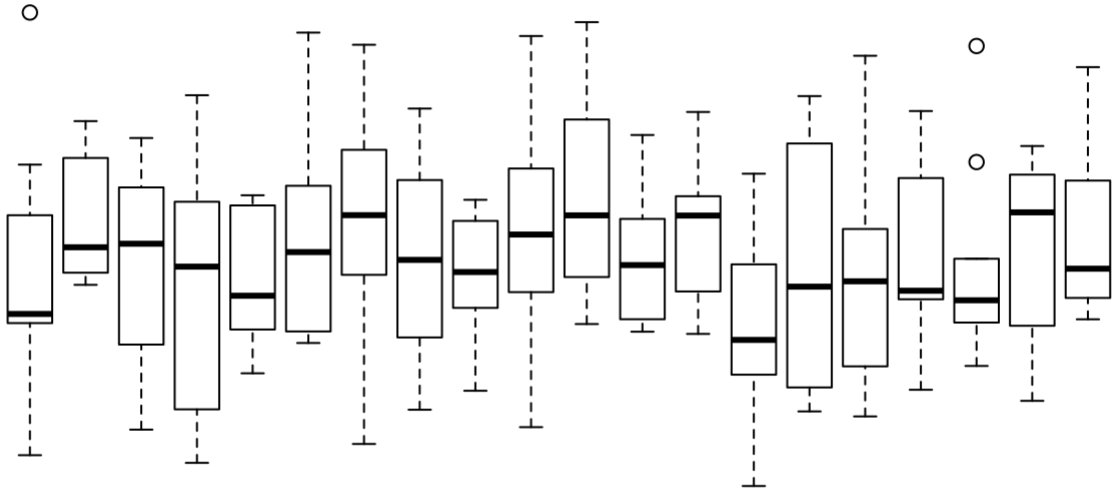
# Data snooping / fishing

> If you look at the data and then decide which hypothesis to test, your p-value is invalid and you are not actually getting the stated Type I error rate.

It's as if you did some large number of tests $M$ (informally)… so if you chose the "most promising looking" test out of $M = 6$ possibilities, you'd have a $25\%$ of making a Type I error! Even though you used $\alpha = 0.05$.

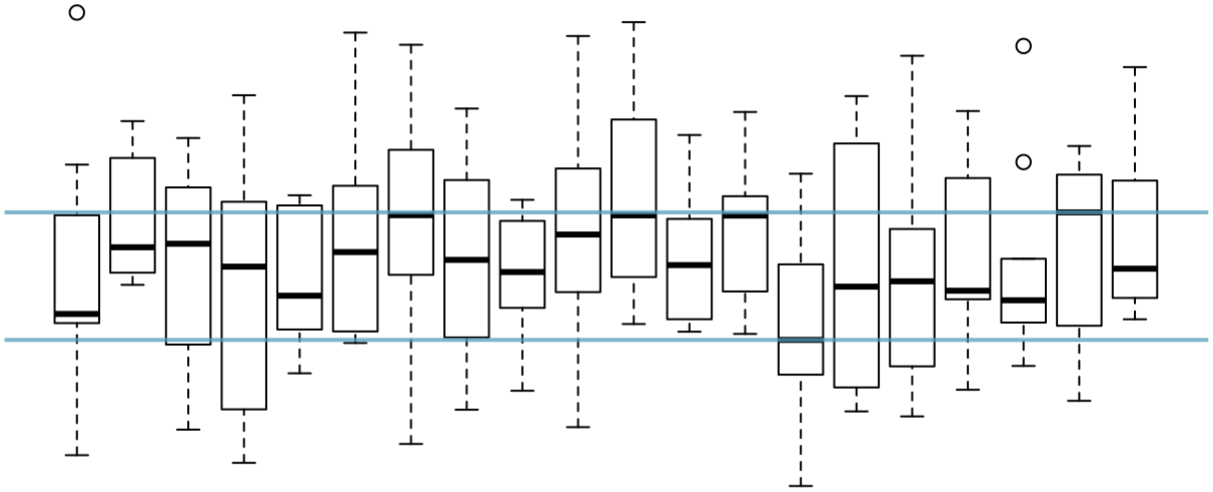
# In pictures

Generate 20 groups of size 10 from $N(0, 1)$.



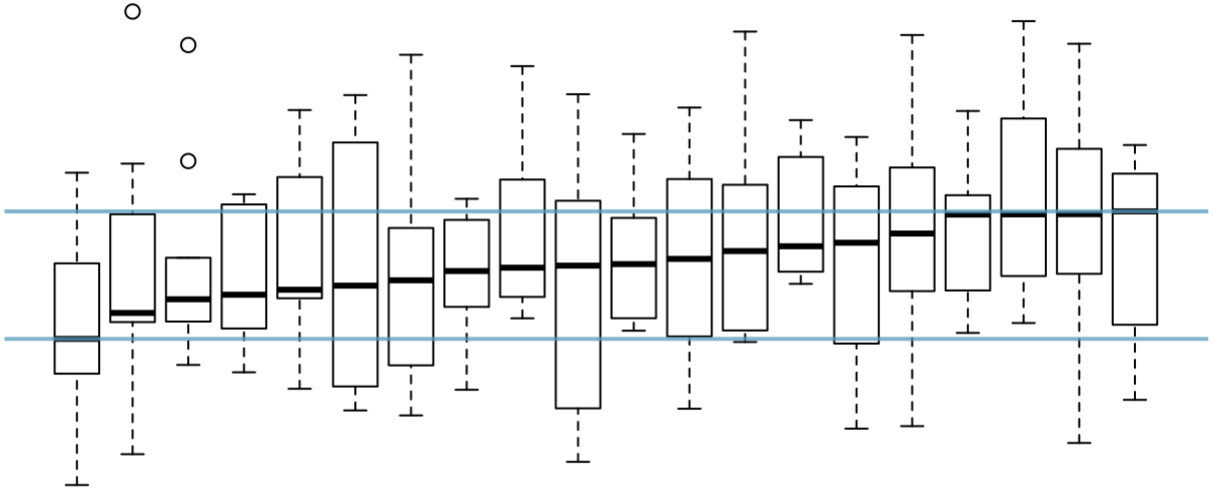
# In pictures

A lot of variation in medians… though all from from $N(0, 1)$.



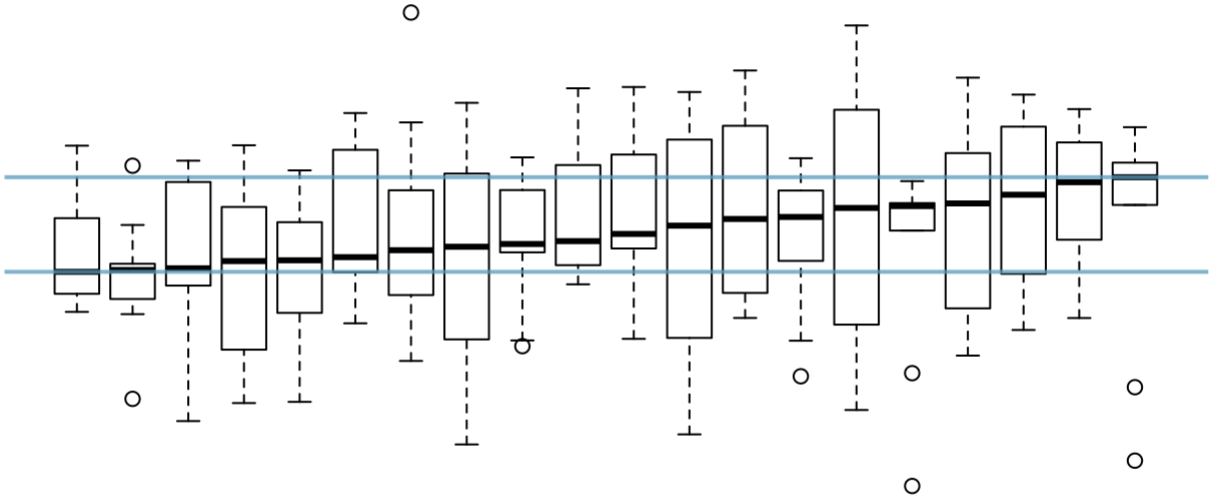
# In pictures

Sort these. Lowest dependably far from highest…



# In pictures (repeat)

Sort these. Lowest dependably far from highest…



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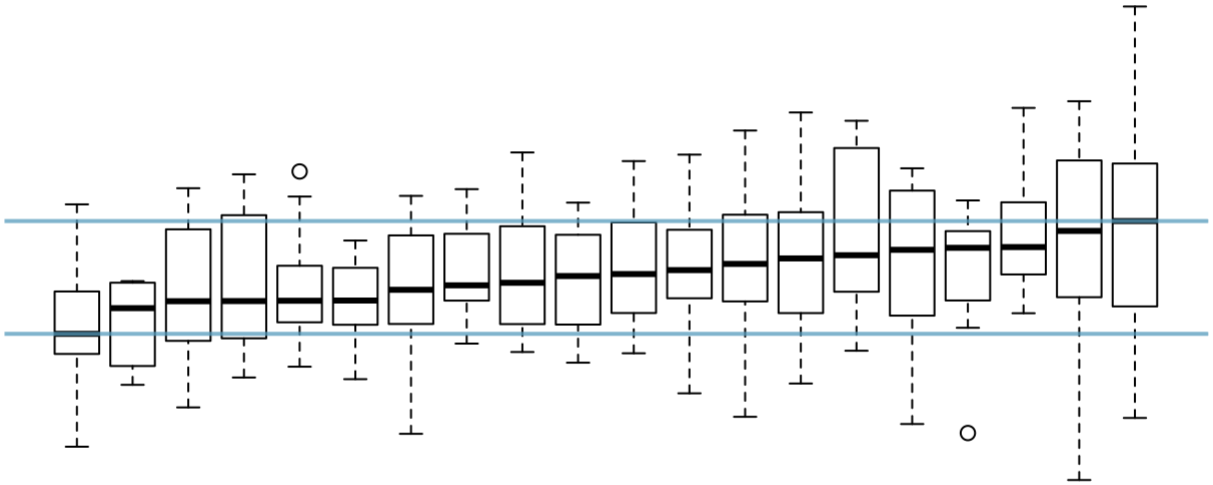
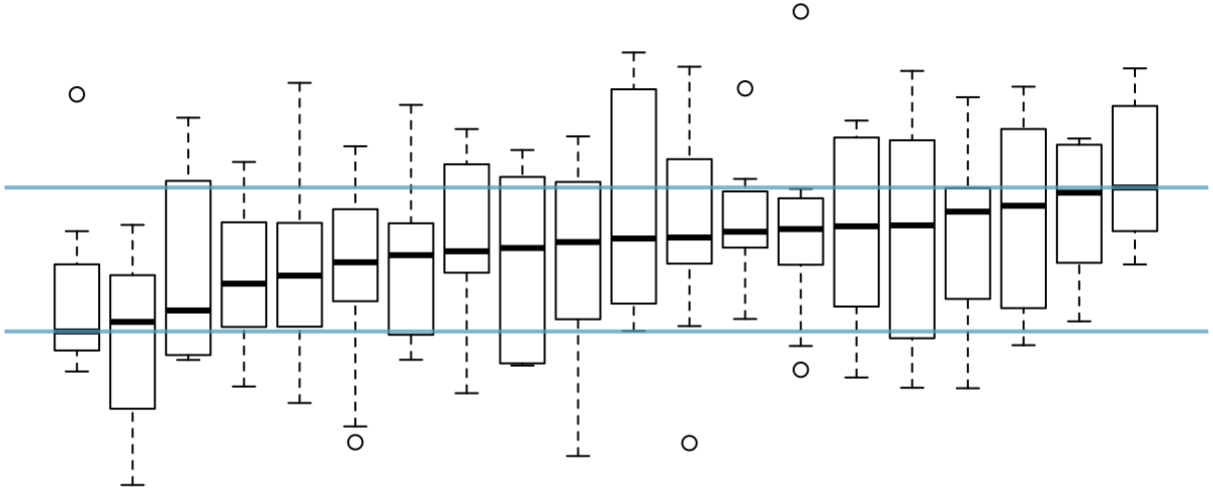


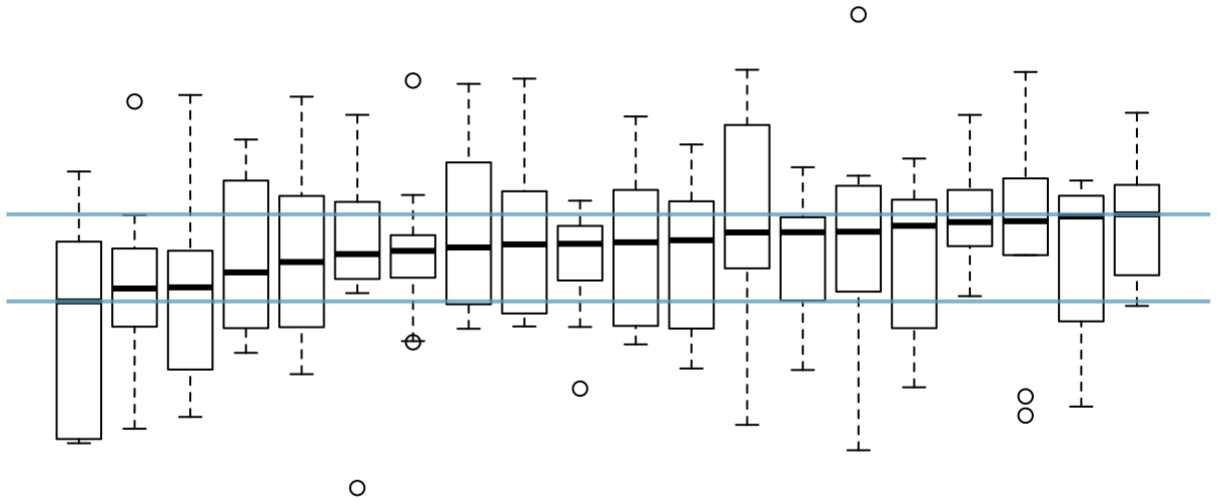
# In pictures (repeat)

Sort these. Lowest dependably far from highest…



# In pictures (repeat)

Sort these. Lowest dependably far from highest…



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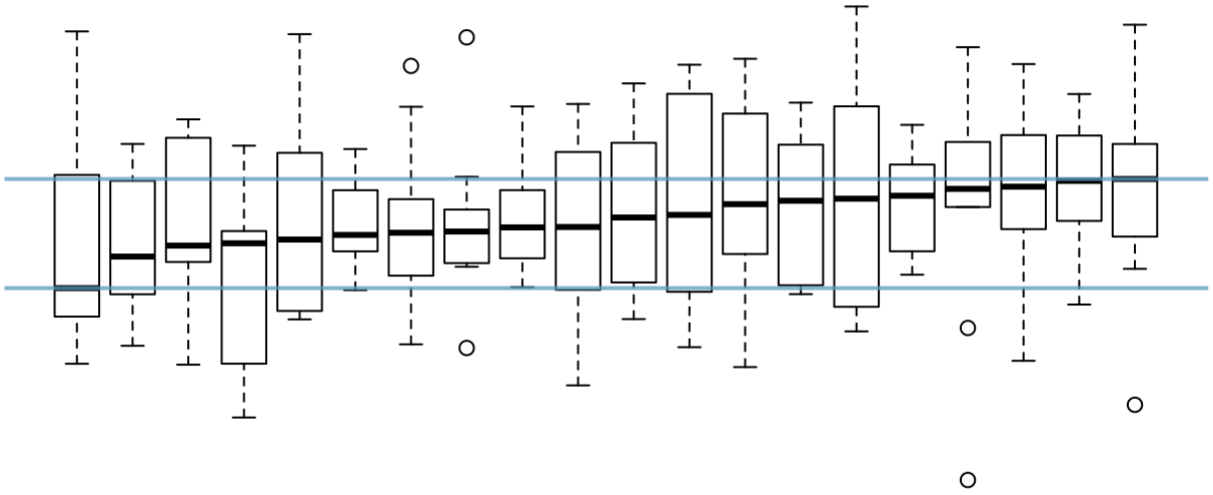


# A simple (but suboptimal) fix

**Bonferroni correction** If you're doing $M$ tests and you want an overall Type I error rate of $\alpha$, then do your individual tests at level $\alpha^* = \frac{\alpha}{M}$.

- works even if tests are dependent
- for $M = 6$, we should do tests with $\alpha^* = 0.05/6$.
- not a great fix since hard to reject a null - i.e., we **lose power**
- Bonferroni is simple but overly conservative
- What if you are doing $M = 100,000$ as in genomics??

# Test all pairs of labs at 0.05/6 level

Compute p-values: e.g., `t.test(red,blue)$p.value`

```
## [1] 0.9529006
```

```
## [1] 0.4620212
```

```
## [1] 0.9529006
```

```
## [1] 0.3863428
```

```
## [1] 0.9583997
```

```
## [1] 0.4285551
```

- none are even close to $0.05/6 \approx 0.008$

# So what should we do?

- after correction for multiple testing, doing all $\binom{k}{2}$ tests can have low power
- sometimes we just want to say that **not all means are equal** without saying which pair it is.
- e.g., lab assignment is *associated* with prelim grades
- not trying to say something specific like lab "Red" is different from lab "Blue"
- We wish to test

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_k$$
$$H_A : \text{ at least one mean is different}$$

This is the goal of **ANOVA** (**AN**alysis **O**f **VA**riance)

# One-way ANOVA

# Outline 2

*Assume: independence, normality and equal variance*

*Model each observation as ``group mean + noise''*

*Compare variability between groups to variability within groups*

*How?*

- *Assuming no difference in group means, measure how between/within group variability should look*
- *See if between/within variability is substantially higher in your data*

*General guideline: be careful about making causal claims from observational data*

# Contexts for using ANOVA

**Observational study**

- draw independent samples of size

$$n_1, \ldots, n_k$$

  from $k \geq 2$ populations.

- Goal: Are the means of the $k$ populations all the same?

- allows us to make inferences about **associations** (as in correlations)

**Controlled experiments**

- draw independent samples then randomly assign subjects to one of $k$ treatment groups

- Goal: Are the means of the $k$ populations all the same?

- allows us to make **causal** inferences

# Question

### Example: Prelim scores in labs

Suppose we reject the null of $\mu_{\text{Green}} = \mu_{\text{Red}}$ in favor of $\mu_{\text{Green}} > \mu_{\text{Red}}$, what can we conclude?

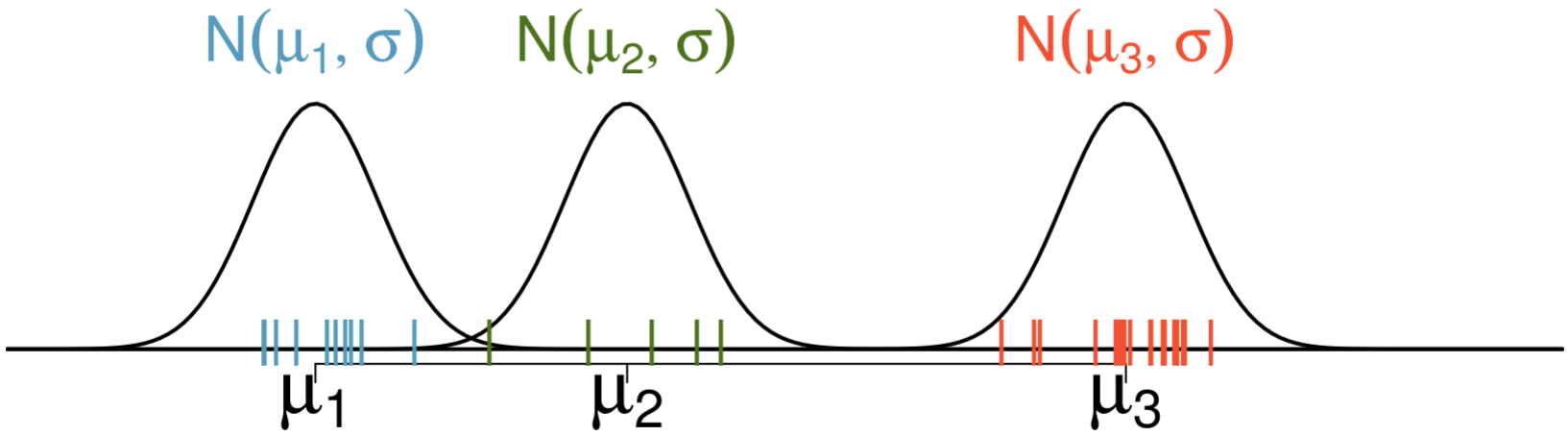
(Confounders?)

# 0. Assumption and ANOVA model

# Assumptions to use ANOVA

- independence (both between groups and within)
- normality
- equal variances

  ***Common estimator for the population***




(groups can only differ by mean shifts)

# Basic ANOVA: model

Let $Y_{ij}$ = response for unit $j$ from group $i$ (e.g., score of student $j$ in lab $i$)

All assumptions incorportated by following linear model: *Assume the effects are additive*

$$
\begin{aligned}
Y_{1j} &= \mu_1 + \epsilon_{1j} \text{ for } j = 1, \ldots, n_1 \\
Y_{2j} &= \mu_2 + \epsilon_{2j} \text{ for } j = 1, \ldots, n_2 \\
&\vdots \\
Y_{kj} &= \mu_k + \epsilon_{kj} \text{ for } j = 1, \ldots, n_k
\end{aligned}
$$

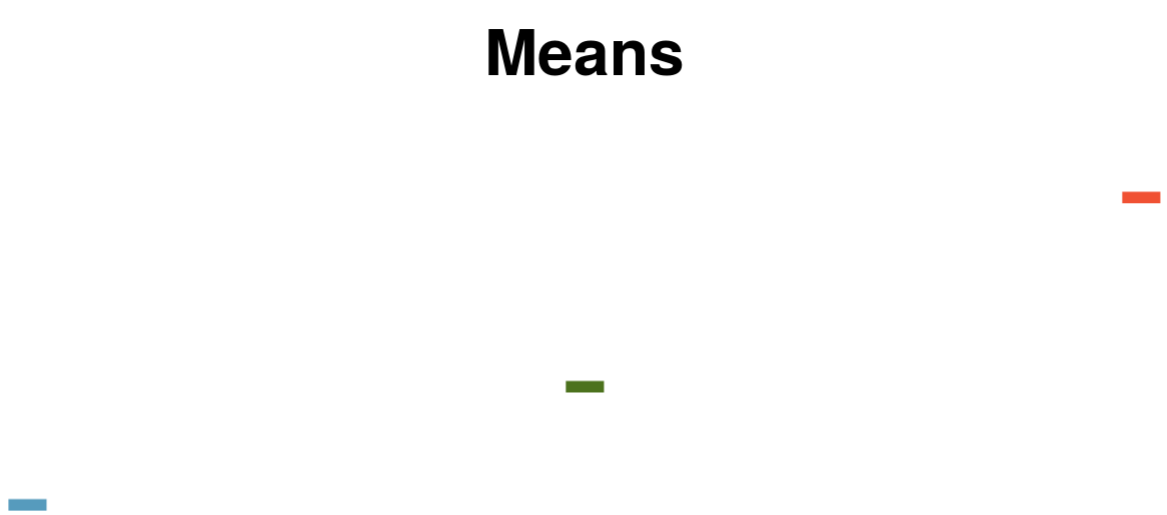
where $\epsilon_{ij} \sim N(0, \sigma)$ are all independent.

> Response = Mean of group + Noise

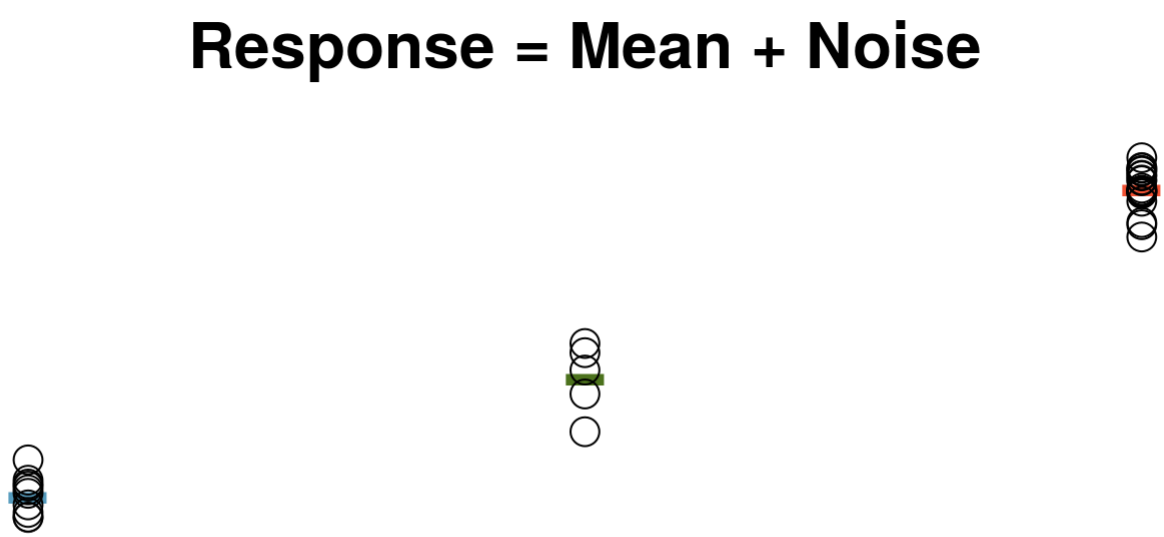
# Basic ANOVA: model in words

- each group (potentially) has its own mean.
- response is simply the mean corrupted by noise
- noise is independent, $N(0, \sigma)$ with same $\sigma$ across all groups

# Basic ANOVA: model in pictures



# Basic ANOVA: model in pictures



# 1. Null and Alternative Hypotheses

# ANOVA

$$H_0 : \mu_1 = \cdots = \mu_k$$

**What null looks like**

# Basic ANOVA: model (alternate notation)

*Some deviation from the grand mean*

$$
\begin{aligned}
Y_{1j} &= \mu + \alpha_1 + \epsilon_{1j} \text{ for } j = 1, \ldots, n_1 \\
Y_{2j} &= \mu + \alpha_2 + \epsilon_{2j} \text{ for } j = 1, \ldots, n_2 \\
&\vdots \\
Y_{kj} &= \mu + \alpha_k + \epsilon_{kj} \text{ for } j = 1, \ldots, n_k
\end{aligned}
$$

In this case, we test whether

$$H_0 : \alpha_1 = \cdots = \alpha_k = 0$$

Think of $\alpha_i$ as how group $i$'s mean differs from $\mu$, i.e. $\alpha_i = \mu_i - \mu$

(We require $\sum_{i=1}^{k} \alpha_i = 0$ so $\mu$ represents the "overall mean")

# One-way fixed effects ANOVA

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \text{ for } j = 1, \ldots, n_i$$

**Notation:**

- $n_i$ is number of units in group $i$
- $Y_{ij}$ is response of unit $j$ in group $i$
- $\mu$ is overall mean response value
- $\alpha_i$ is effect on mean response due to group $i$
- $\epsilon_{ij}$ random error for unit $j$ of group $i$

# Fixed effects vs. random effects

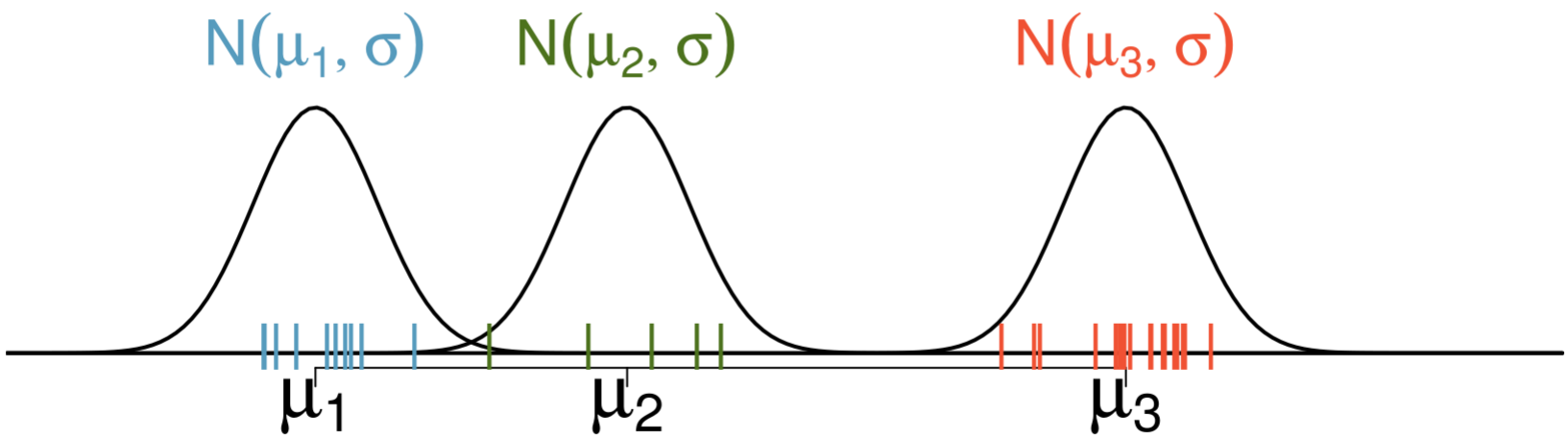
**Fixed effects ANOVA** (our focus) - use when we care about the specific $k$ groups present in sample - e.g., we care about these particular four labs

**Random effects ANOVA** (in BTRY 6020) - use when these specific $k$ groups are not themselves of intrinsic interest, but rather have been chosen to represent a larger population of potential groups - e.g., company wants to know if employee interviews influenced by who's interviewing. SRS of 5 interviewers (out of, say, 100s) and has each interview 10 applicants

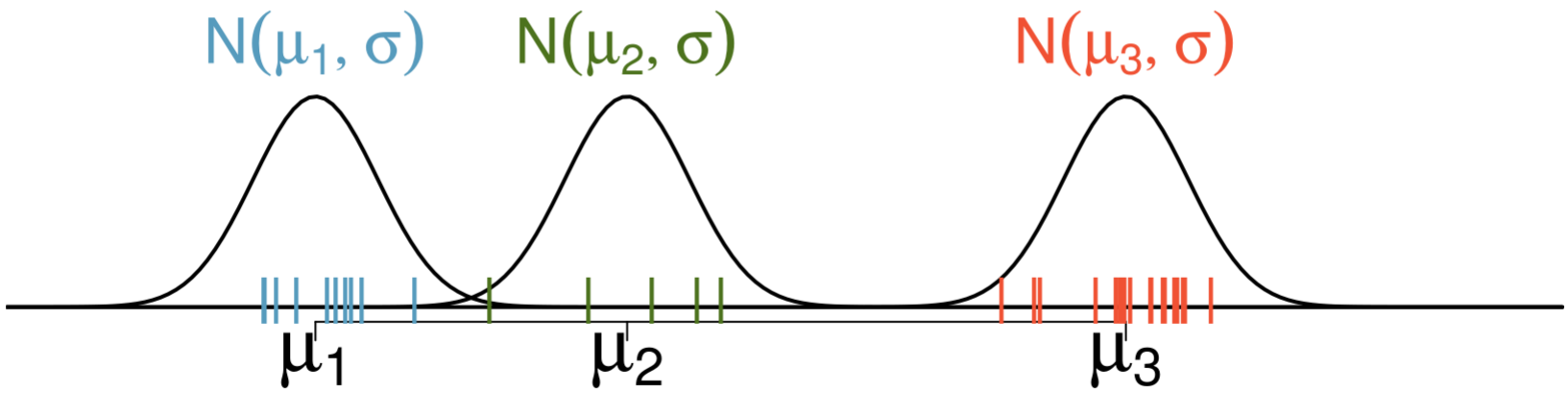
# 2. Measure within and between group variability

# Principal idea behind ANOVA

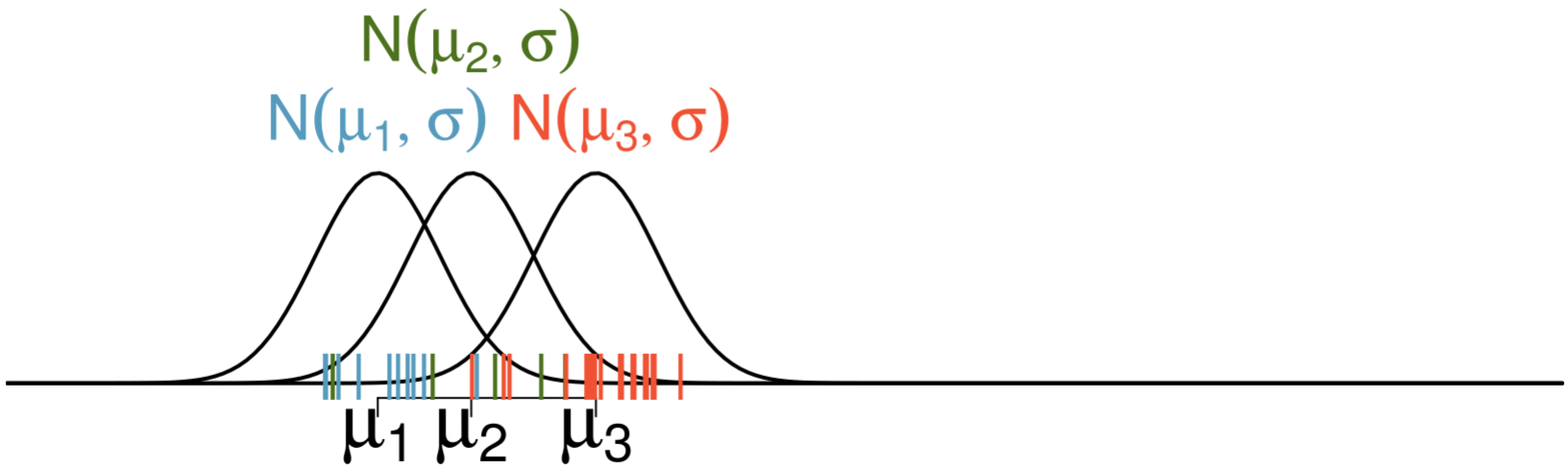
Compare **variability between** the $k$ estimated group-specific means to a pooled estimate of **within-group variability**



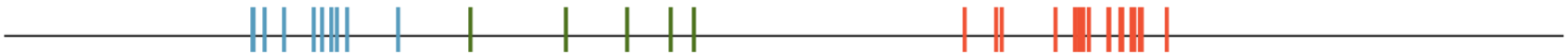
# Within group variance smaller than between group variance



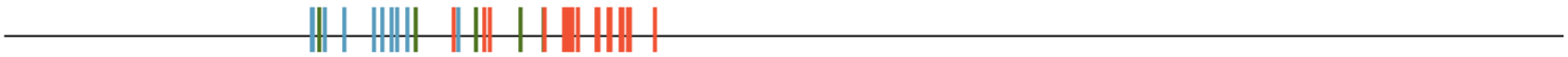
# Within group variance bigger than between group variance



# Within group variance smaller than between group variance



# Within group variance bigger than between group variance



# Estimation in fixed effects model

**Model:** $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$

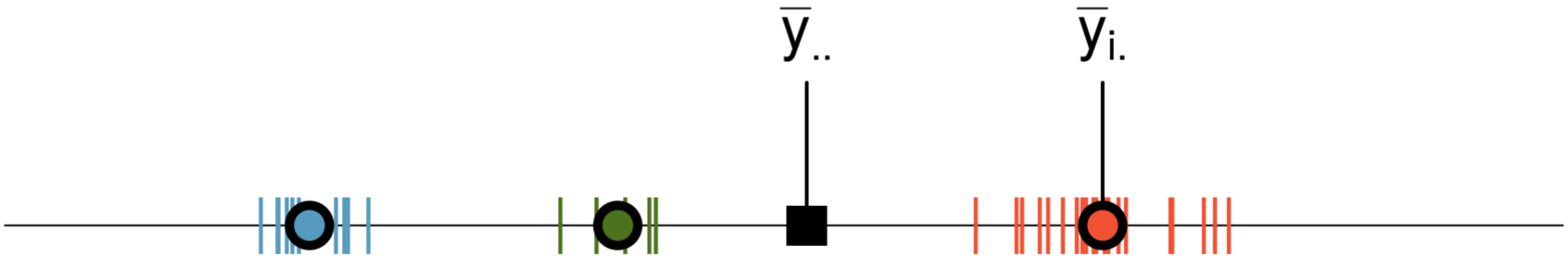
**Observed data**

$y_{ij}$ for $j = 1, \ldots, n_i$ (and $i = 1, \ldots, k$)

Notation:

- **group-specific sample mean:** $\bar{y}_{i\cdot} = \frac{1}{n_i} \sum_{j=1}^{n_i} y_{ij}$
- **overall sample mean:** $\bar{y}_{\cdot\cdot} = \frac{1}{n_{tot}} \sum_{i=1}^{k} \sum_{j=1}^{n_i} y_{ij}$

(here $n_{tot} = \sum_{i=1}^{k} n_i$)

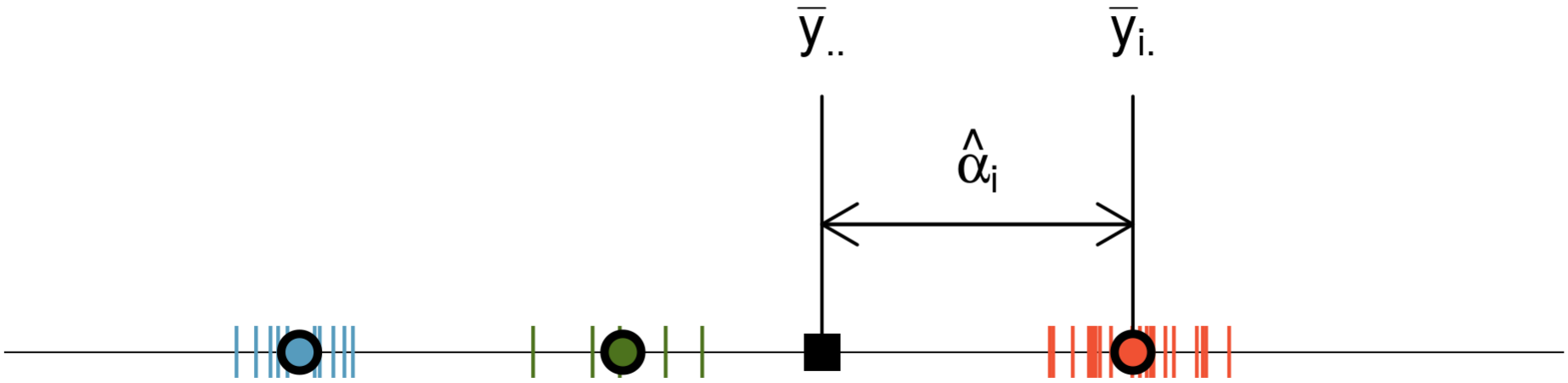

$\bar{y}_{..}$
$\bar{y}_{i.}$

# Estimation in fixed effects model

**Model:** $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$

**Obvious estimates:**

$$\hat{\mu} = \bar{y}_{..} \qquad \hat{\alpha}_i = \bar{y}_{i.} - \bar{y}_{..}$$

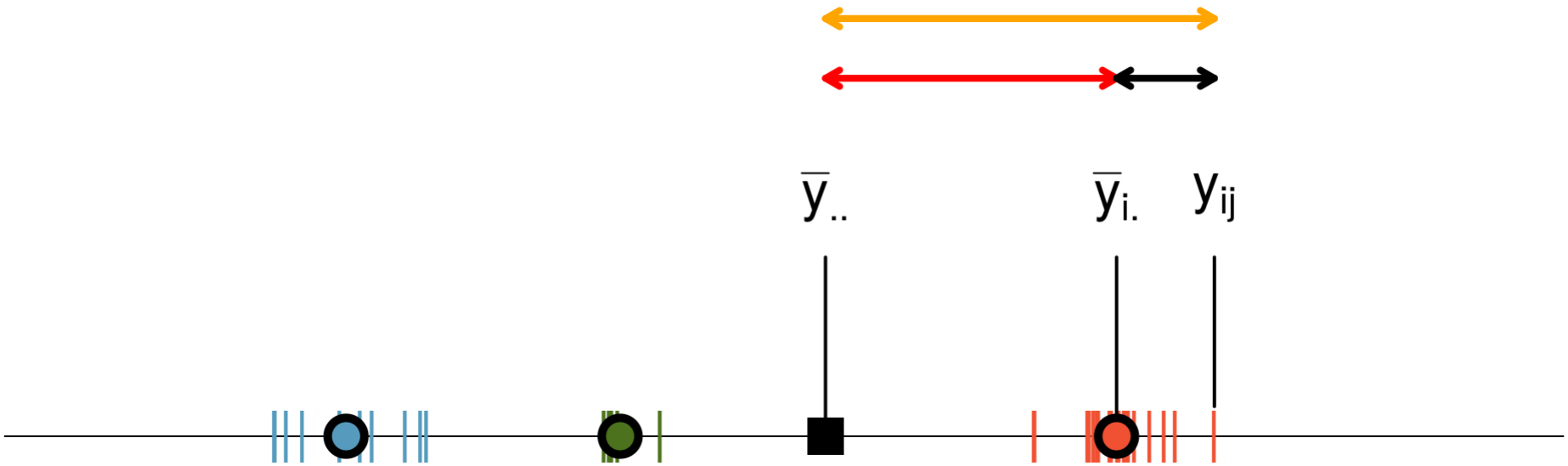


# ANOVA decomposition

*Variability between groups (systematic variation)*

*Variability within groups (natural variation)*

$$y_{ij} - \bar{y}_{..} = (y_{ij} - \bar{y}_{i.}) + (\bar{y}_{i.} - \bar{y}_{..})$$




$$\sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{..})^2 = \sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{i.})^2 + \sum_{i=1}^{k} n_i(\bar{y}_{i.} - \bar{y}_{..})^2$$

*Should be a^2 + b^2 - 2ab but the last term cancels out*

# ANOVA decomposition

$$\sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij}-\bar{y}_{..})^2 = \sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij}-\bar{y}_{i.})^2 + \sum_{i=1}^{k} n_i(\bar{y}_{i.}-\bar{y}_{..})^2$$

$$\text{tss} = \text{sse} + \text{ssb}$$

> Total sum of squares = sum of squares within group + sum of squares between groups

- **tss:** variability about the overall mean
- recognize $tss/(n_{tot}-1)$?

# 2a. Within group variability

sse (sum of squares of errors)

mse (mean squares of errors)

# sse (as pooled error variance)

Regardless of whether $H_0$ or $H_A$ holds, an unbiased estimate for $\sigma^2$ given by

$$\frac{1}{n_{tot}-k}\sum_{i=1}^{k}\sum_{j=1}^{n_i}(y_{ij}-\bar{y}_{i\cdot})^2=\frac{sse}{n_{tot}-k}$$

Recall **sample variance for group $i$**:

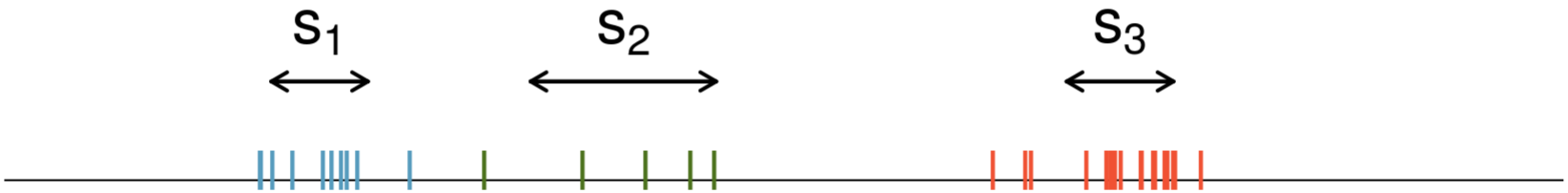
$$s_i^2=\frac{1}{n_i-1}\sum_{j=1}^{n_i}(y_{ij}-\bar{y}_{i\cdot})^2$$

$$"\ mse\ "=\frac{sse}{n_{tot}-k}=\frac{1}{n_{tot}-k}\sum_{i=1}^{k}(n_i-1)s_i^2$$

# mse (as pooled error variance)

$$mse = \frac{sse}{n_{tot} - k} = \frac{1}{n_{tot} - k} \sum_{i=1}^{k} (n_i - 1) s_i^2$$

- weighted average of groups' sample variances
- bigger groups get more weight



# 2b. Between group variability

ssb (sum of squares between groups)

msb (mean squares between groups)

# ssb: Between groups variability

ANOVA decomposition suggests that

$$ssb = \sum_{i=1}^{k} n_i(\bar{y}_{i\cdot} - \bar{y}_{\cdot\cdot})^2$$

is a way to measure variability **between groups**.

How much *on average* does a group vary from grand mean?

$$msb = \frac{ssb}{k-1}$$

Under $H_0$:

- should be small (purely due to random fluctuations of noise)
- in particular, would expect $msb \approx mse$

# 3. Compare between to within

- Test Statistic: MSB/MSE
- Under $H_0$, follows **F distribution** with appropriate df
- Reject $H_0$ if test statistic **is large**

# F statistic

Under $H_0$, we expect $msb \approx mse$. More precisely…

Let $MSB$ and $MSE$ be the random variables (of which msb and mse are realizations)

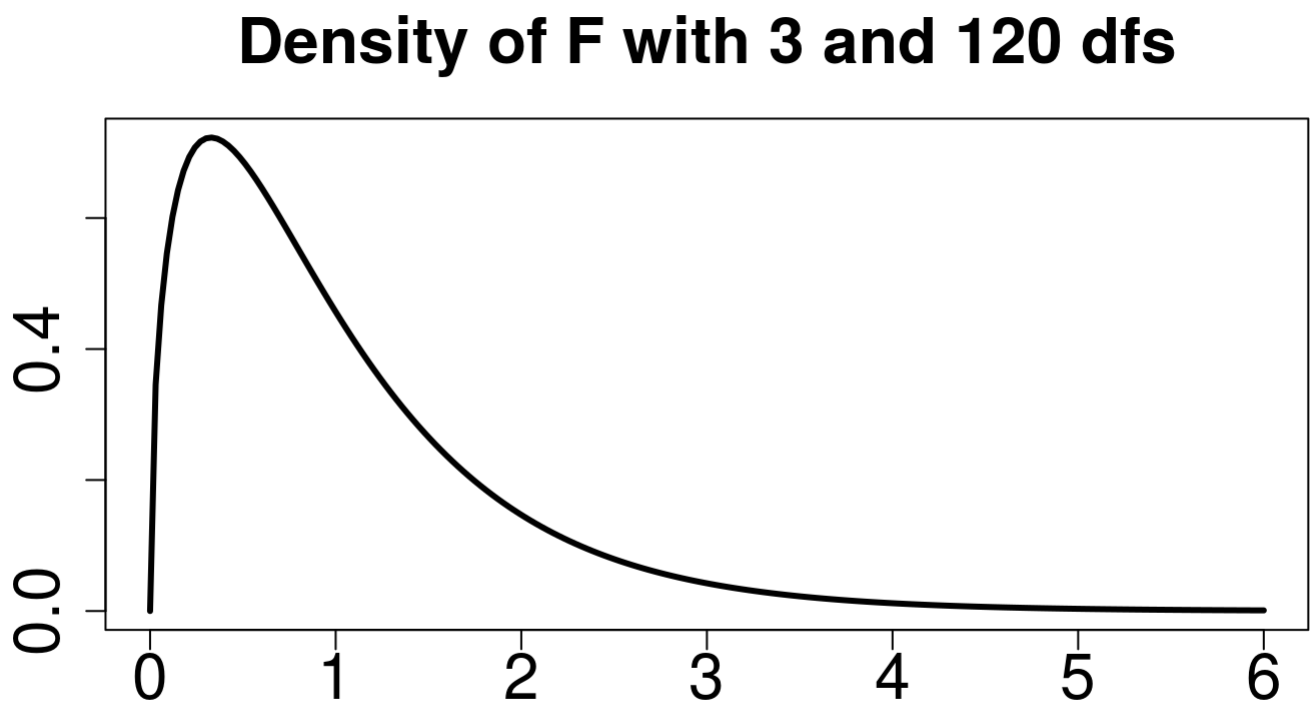
Under $H_0$, the statistic

$$\frac{MSB}{MSE} \sim F_{k-1, n_{tot}-k},$$

that is, it has an $F$ distribution with $k-1$ and $n_{tot} - k$ degrees of freedom.

# F distribution

**F** stands for Fisher



# Rejecting null

Under $H_0$, the statistic

$$\frac{MSB}{MSE} \sim F_{k-1,n_{tot}-k},$$

If $\frac{msb}{mse}$ is "larger than expected under null", we reject $H_0$ in favor of $H_A$.

The **ANOVA p-value** is given by

$$P\left(F_{k-1,n_{tot}-k} > \frac{msb}{mse}\right)$$

# The ANOVA Table

| Source of variability | Degrees of freedom | Sum of squares | Mean square | F statistic | p-value |
|---|---|---|---|---|---|
| Between | $k-1$ | ssb | $msb = \frac{ssb}{k-1}$ | $\frac{msb}{mse}$ | $P\left(F_{k-1,n_{tot}-k} > \frac{msb}{mse}\right)$ |
| Error (within) | $n_{tot}-k$ | sse | $mse = \frac{sse}{n_{tot}-k}$ | | |
| Total | $n_{tot}-1$ | tss | | | |

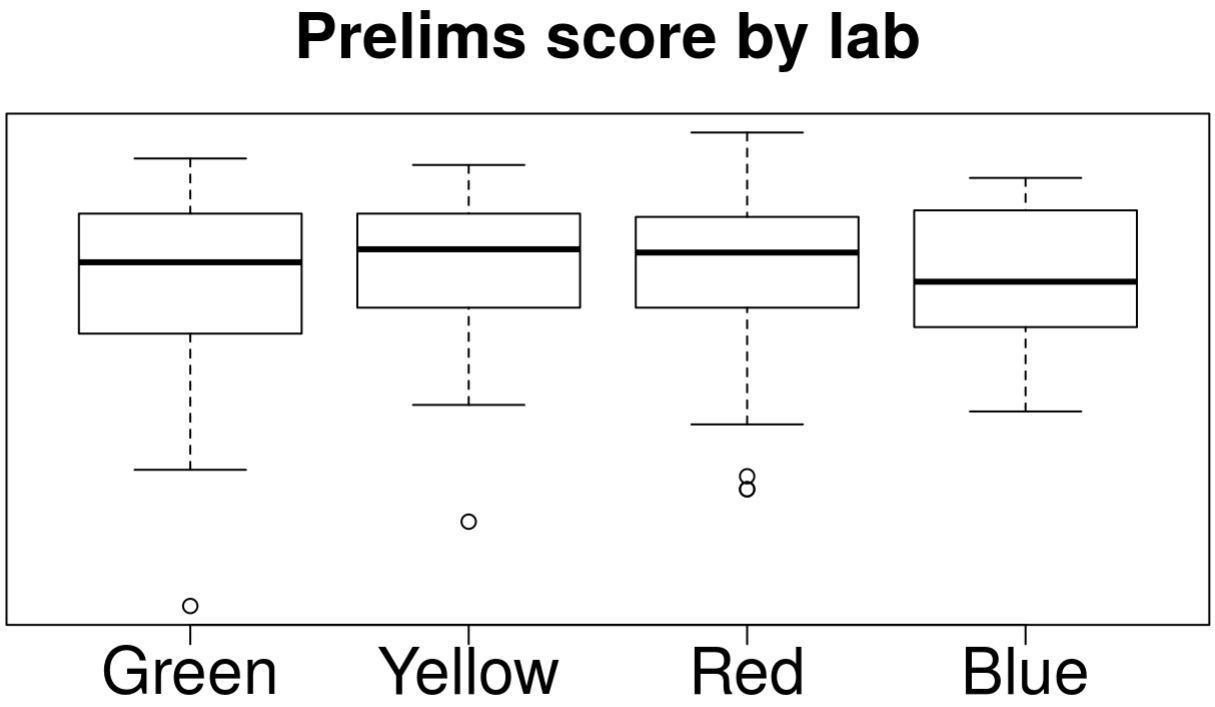
# 4. Check Assumptions

Independence: revisit sampling strategy used in data collection

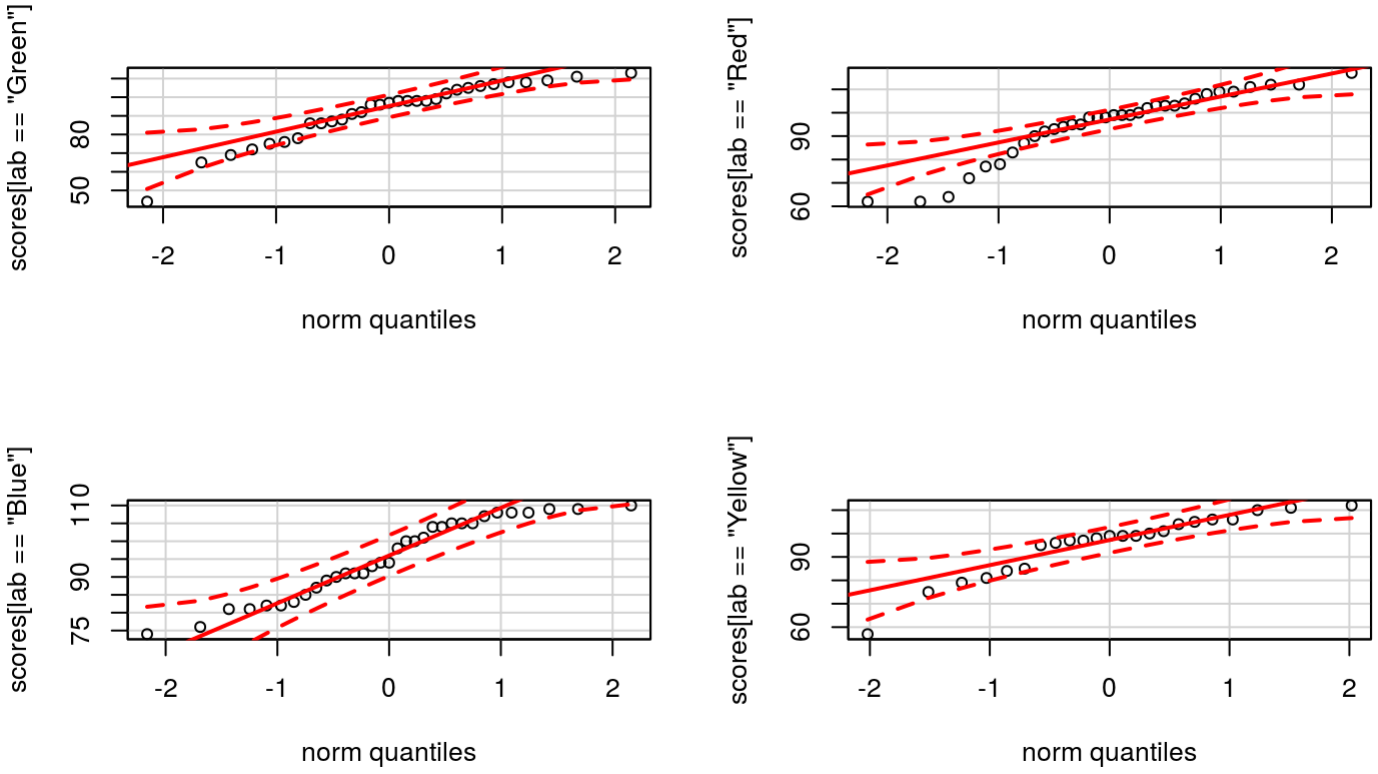
Normality: Q-Q plots, histograms

Equal variance: side-by-side boxplots, Levene's Test (to be covered in lab this week)

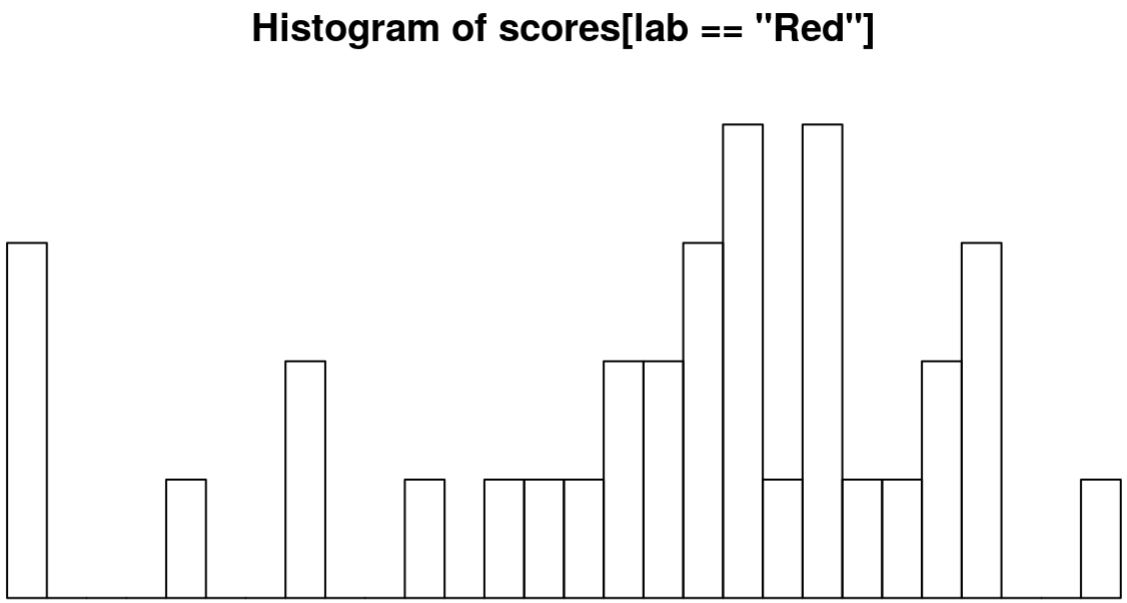
# Back to the labs question



# Assumptions met?



# A look at Red lab



# Importance of assumptions for ANOVA

- **independence** is very important
- **normality** is less important especially if sample sizes are close to equal (and not too small)
- **equal** $\sigma_i$ assumption is crucial
- watch out for **outliers** (can repeat analysis with removed and see if there's a difference)

# Let's proceed…

```
fit = aov(scores ~ lab)
summary(fit)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## lab           3    203   67.73   0.359  0.783
## Residuals   117  22059  188.54
```

# Rerun after removing outliers

```
fit2 = aov(scores[-ii]~lab[-ii])
summary(fit2)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## lab[-ii]      3    334   111.4   0.853  0.468
## Residuals   112  14631   130.6
```

Still no evidence against null.

# 5. R commands

### aov

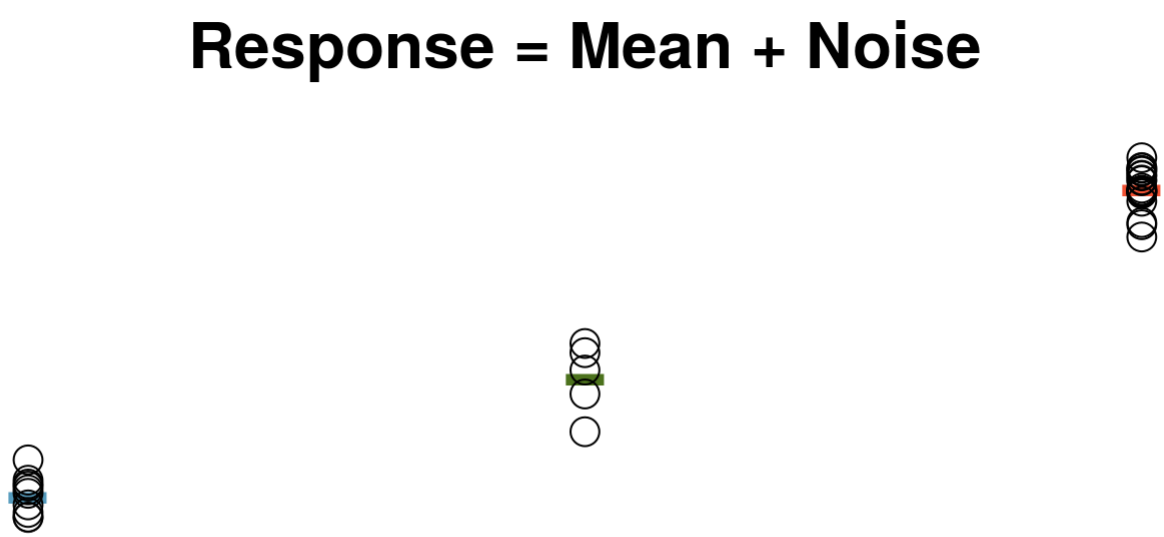
```
fit = aov(scores~lab)
summary(fit)
```

### lm

```
scores.lm = lm(scores ~ lab)
anova(scores.lm)
```

# Finishing up ANOVA

# Basic ANOVA: model in pictures



# ANOVA

Want to test

$$H_0 : \mu_1 = \cdots = \mu_k$$

versus $H_A$ : means are not all the same

# Basic ANOVA: model

$$
\begin{aligned}
Y_{1j} &= \mu + \alpha_1 + \epsilon_{1j} \text{ for } j = 1, \ldots, n_1 \\
Y_{2j} &= \mu + \alpha_2 + \epsilon_{2j} \text{ for } j = 1, \ldots, n_2 \\
&\vdots \\
Y_{kj} &= \mu + \alpha_k + \epsilon_{kj} \text{ for } j = 1, \ldots, n_k
\end{aligned}
$$

In this case, we test whether $H_0 : \alpha_1 = \cdots = \alpha_k = 0$

Think of $\alpha_i$ as how group $i$'s mean differs from $\mu$, i.e. $\alpha_i = \mu_i - \mu$

(We require $\sum_{i=1}^{k} \alpha_i = 0$ so $\mu$ represents the "overall mean")

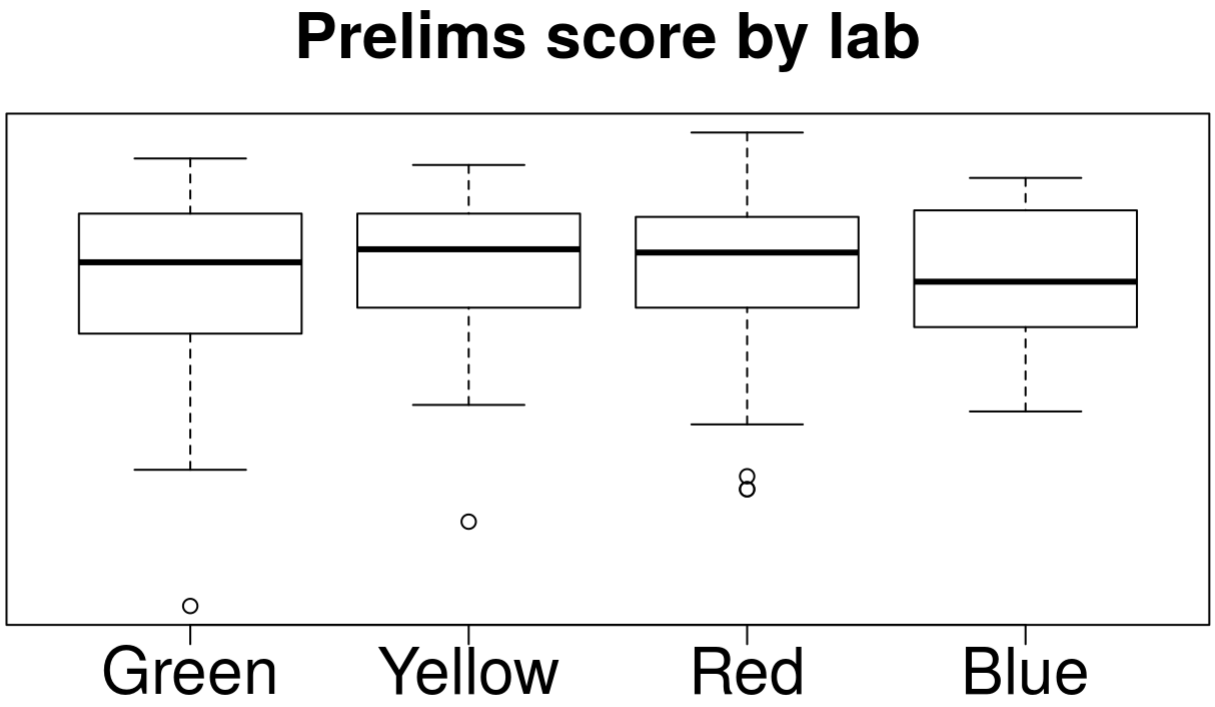
# One-way fixed effects ANOVA

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \text{ for } j = 1, \ldots, n_i$$

**Notation:**

- $n_i$ is number of units in group $i$
- $Y_{ij}$ is response of unit $j$ in group $i$
- $\mu$ is overall mean response value
- $\alpha_i$ is **effect** on mean response due to group $i$
- $\epsilon_{ij} \sim N(0, \sigma)$ random error for unit $j$ of group $i$

# Back to the labs question



# In R...

```
scores.lm = lm(scores ~ lab)
anova(scores.lm)
```

```
## Analysis of Variance Table
##
## Response: scores
##            Df  Sum Sq Mean Sq F value Pr(>F)
## lab         3   203.2  67.729  0.3592 0.7826
## Residuals 117 22059.0 188.539
```

# Rerun after removing outliers

```
scores2.lm = lm(scores[-ii] ~ lab[-ii])
anova(scores2.lm)
```

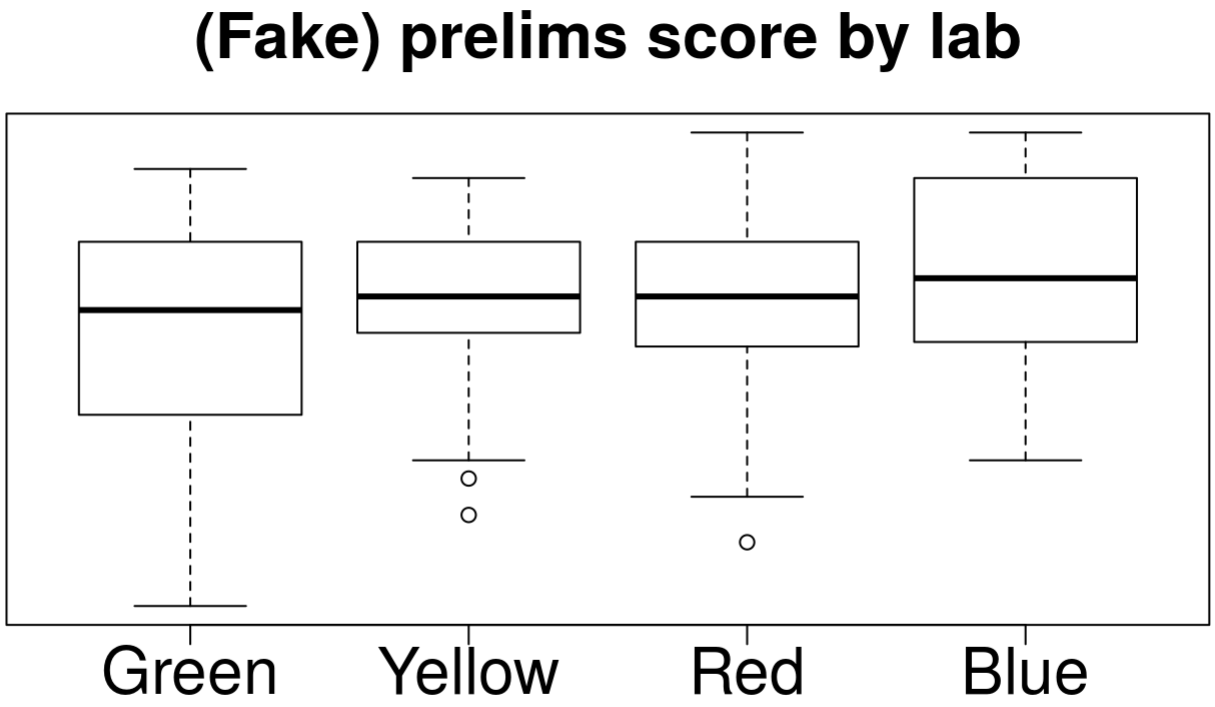
```
## Analysis of Variance Table
##
## Response: scores[-ii]
##            Df  Sum Sq Mean Sq F value Pr(>F)
## lab[-ii]    3   334.1  111.37  0.8526 0.4681
## Residuals 112 14630.6  130.63
```

Still no evidence against null.

# 6. If $H_0$ rejected, which pairs differ in mean?

- Can go back to pairwise t-tests, **with multiple testing correction (using, say, Bonferroni)**
- Can use **Tukey's Honest Significance Difference (HSD) test**

# Suppose the data looked like this



# ANOVA on fake data

```
scores3.lm = lm(scores~lab, data = dat2)
anova(scores3.lm)
```

```
## Analysis of Variance Table
##
## Response: scores
##            Df  Sum Sq Mean Sq F value  Pr(>F)
## lab         3  1121.3  373.77  2.8613 0.04007 *
## Residuals 112 14630.6  130.63
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- At $\alpha = 0.05$ level, we reject $H_0$ in favor of $H_A$.

# Which pair(s) are different?

- can do pairwise tests as long as we correct for multiple testing
- in this context, there's something better than Bonferroni…
- **Tukey Honest Significant Differences** (Tukey HSD)
- takes into account that we are looking at $\binom{k}{2}$ comparisons

# In R

```
library(multcomp)
hsd <- glht(scores3.lm, linfct = mcp(lab = "Tukey"))
summary(hsd)
```

[Output on next slide.]

# In R

```
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: lm(formula = scores ~ lab, data = dat2)
##
## Linear Hypotheses:
##                      Estimate Std. Error t value Pr(>|t|)
## Yellow - Green == 0    3.294      3.208   1.027   0.7336
## Red - Green == 0       4.325      2.927   1.477   0.4537
## Blue - Green == 0      8.370      2.883   2.903   0.0225 *
## Red - Yellow == 0      1.031      3.186   0.324   0.9882
## Blue - Yellow == 0     5.076      3.146   1.613   0.3746
## Blue - Red == 0        4.045      2.859   1.415   0.4920
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

# Final note

- can sometimes reject $H_0 : \mu_1 = \mu_2 = \cdots = \mu_k$ and yet no pairwise test would reject!
- ANOVA pools information across all groups and tries to answer a more general question