Analysis of variance

# Packages

library(tidyverse)
library(smmr)
library(PMCMRplus)

# Jumping rats

- Link between exercise and healthy bones (many studies).
- Exercise stresses bones and causes them to get stronger.
- Study (Purdue): effect of jumping on bone density of growing rats.
- 30 rats, randomly assigned to 1 of 3 treatments:
  - No jumping (control)
  - Low-jump treatment (30 cm)
  - High-jump treatment (60 cm)
- 8 weeks, 10 jumps/day, 5 days/week.
- Bone density of rats (mg/cm<sup>3</sup>) measured at end.

# Jumping rats 2/2

- See whether larger amount of exercise (jumping) went with higher bone density.
- Random assignment: rats in each group similar in all important ways.
- So entitled to draw conclusions about cause and effect.

Values separated by spaces:

my\_url <- "http://ritsokiguess.site/datafiles/jumping.txt"
rats <- read\_delim(my\_url," ")</pre>

## The data (some random rows) rats %>% slice\_sample(n=10)

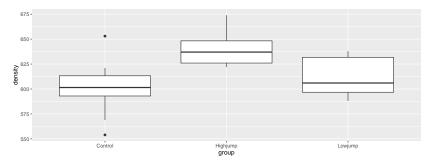
# 4	A tibble:	10 x 2			
group		density			
	<chr></chr>	<dbl></dbl>			
1	Control	621			
2	Lowjump	607			
3	Lowjump	635			
4	Lowjump	632			
5	Highjump	643			
6	Control	611			
7	Highjump	650			
8	Lowjump	588			
9	Lowjump	594			
10	Highjump	674			

#### rats

# A tibble: 30 x 2

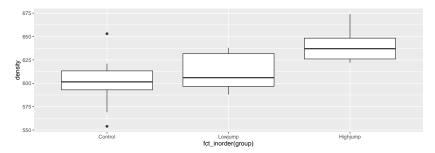
#### Boxplots

#### ggplot(rats, aes(y=density, x=group)) + geom\_boxplot()



Or, arranging groups in data (logical) order

ggplot(rats, aes(y=density, x=fct\_inorder(group))) +
geom\_boxplot()



# Analysis of Variance

- Comparing > 2 groups of independent observations (each rat only does one amount of jumping).
- Standard procedure: analysis of variance (ANOVA).
- Null hypothesis: all groups have same mean.
- Alternative: "not all means the same", at least one is different from others.

# Testing: ANOVA in R

```
rats.aov <- aov(density~group,data=rats)
summary(rats.aov)</pre>
```

Df Sum Sq Mean Sq F value Pr(>F) group 2 7434 3717 7.978 0.0019 \*\* Residuals 27 12579 466 ---Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 '

- Usual ANOVA table, small P-value: significant result.
- Conclude that the mean bone densities are not all equal.
- Reject null, but not very useful finding.

# Which groups are different from which?

- ANOVA really only answers half our questions: it says "there are differences", but doesn't tell us which groups different.
- One possibility (not the best): compare all possible pairs of groups, via two-sample t.
- First pick out each group:

```
rats %>% filter(group=="Control") -> controls
rats %>% filter(group=="Lowjump") -> lows
rats %>% filter(group=="Highjump") -> highs
```

#### Control vs. low

t.test(controls\$density, lows\$density)

```
Welch Two Sample t-test
```

data: controls\$density and lows\$density
t = -1.0761, df = 16.191, p-value = 0.2977
alternative hypothesis: true difference in means is not equ
95 percent confidence interval:
 -33.83725 11.03725

sample estimates:

mean of x mean of y

601.1 612.5

No sig. difference here.

# Control vs. high

t.test(controls\$density, highs\$density)

```
Welch Two Sample t-test
```

```
data: controls$density and highs$density
t = -3.7155, df = 14.831, p-value = 0.002109
alternative hypothesis: true difference in means is not equ
95 percent confidence interval:
-59.19139 -16.00861
sample estimates:
mean of x mean of y
601.1 638.7
```

These are different.

# Low vs. high

t.test(lows\$density, highs\$density)

```
Welch Two Sample t-test
```

data: lows\$density and highs\$density
t = -3.2523, df = 17.597, p-value = 0.004525
alternative hypothesis: true difference in means is not equ
95 percent confidence interval:
 -43.15242 -9.24758
sample estimates:
mean of x mean of y
 612.5 638.7

These are different too.

- We just did 3 tests instead of 1.
- So we have given ourselves 3 chances to reject  $H_0$ : all means equal, instead of 1.
- Thus  $\alpha$  for this combined test is not 0.05.

# John W. Tukey

American statistician, 1915–2000
Big fan of exploratory data analysis
Popularized boxplot
Invented "honestly significant differences"
Invented jackknife estimation
Coined computing term "bit"
Co-inventor of Fast Fourier

Transform

# Honestly Significant Differences

- Compare several groups with one test, telling you which groups differ from which.
- Idea: if all population means equal, find distribution of highest sample mean minus lowest sample mean.
- Any means unusually different compared to that declared significantly different.

```
Tukey on rat data
rats.aov <- aov(density~group, data = rats)
summary(rats.aov)</pre>
```

Df Sum Sq Mean Sq F value Pr(>F) group 2 7434 3717 7.978 0.0019 \*\* Residuals 27 12579 466 ----Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.05 '.' 0.1 ' ' 1 TukeyHSD(rats.aov)

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

```
Fit: aov(formula = density ~ group, data = rats)
```

\$group

diff lwr upr padj

# Why Tukey's procedure better than all t-tests

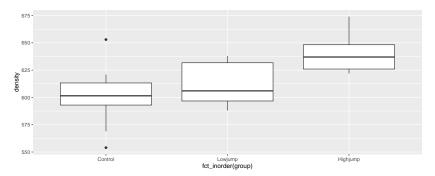
Look at P-values for the two tests:

Comparison	Tukey	t-tests
Highjump-Control	0.0016	0.0021
Lowjump-Control	0.4744	0.2977
Lowjump-Highjump	0.0298	0.0045

- Tukey P-values (mostly) higher.
- Proper adjustment for doing three t-tests at once, not just one in isolation.

# Checking assumptions

ggplot(rats,aes(y = density, x = fct\_inorder(group)))+
geom\_boxplot()



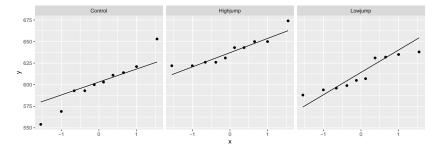
#### Assumptions:

Normally distributed data within each group

with equal group SDs.

## Normal quantile plots by group

ggplot(rats, aes(sample = density)) + stat\_qq() +
stat\_qq\_line() + facet\_wrap( ~ group)



# The assumptions

- Normally-distributed data within each group
- Equal group SDs.
- These are shaky here because:
  - control group has outliers
  - highjump group appears to have less spread than others.
- Possible remedies (in general):
  - Transformation of response (usually works best when SD increases with mean)
  - If normality OK but equal spreads not, can use Welch ANOVA. (Regular ANOVA like pooled t-test; Welch ANOVA like Welch-Satterthwaite t-test.)
  - Can also use Mood's Median Test (see over). This works for any number of groups.

# Mood's median test here

- Find median of all bone densities, regardless of group
- Count up how many observations in each group above or below overall median
- Test association between group and being above/below overall median, using chi-squared test.
- Actually do this using median\_test:

median\_test(rats, density, group)

```
$grand_median
[1] 621.5
```

\$table

above						
group	above	below				
Control	1	9				
Highjump	10	0				
τ	1	<u> </u>				

## Comments

- No doubt that medians differ between groups (not all same).
   This test is equivalent of F-test, not of Tukey.
- To determine which groups differ from which, can compare all possible pairs of groups via (2-sample) Mood's median tests, then adjust P-values by multiplying by number of 2-sample Mood tests done (Bonferroni):

#### pairwise\_median\_test(rats, density, group)

#	# A tibble: 3 x 4						
	g1	g2	p_value	adj_p_value			
	<chr></chr>	<chr></chr>	<dbl></dbl>	<dbl></dbl>			
1	Control	Highjump	0.000148	0.000443			
2	Control	Lowjump	0.371	1			
3	Highjump	Lowjump	0.371	1			

Now, lowjump-highjump difference no longer significant.

# Welch ANOVA

- For these data, Mood's median test probably best because we doubt both normality and equal spreads.
- When normality OK but spreads differ, Welch ANOVA way to go.

# Welch ANOVA done by oneway.test as shown (for illustration):

oneway.test(density~group, data=rats)

One-way analysis of means (not assuming equal variances

data: density and group F = 8.8164, num df = 2.000, denom df = 17.405, p-value = 0

P-value very similar, as expected.

Appropriate Tukey-equivalent here called Games-Howell.

Lives in package PMCMRplus. Install first.

gamesHowellTest(density ~ factor(group), data = rats)

Control Highjump Highjump 0.0056 -Lowjump 0.5417 0.0120

## Deciding which test to do

For two or more samples:

