## 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.
$\rightarrow$ 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 $\left(\mathrm{mg} / \mathrm{cm}^{3}\right)$ 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.


## Reading the data

Values separated by spaces:
my_url <- "http://ritsokiguess.site/datafiles/jumping.txt" rats <- read_delim(my_url," ")

## The data (some random rows)

## rats \%>\% slice_sample(n=10)

\# A tibble: 10 x 2
group density
<chr> <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)
```



- Usual ANOVA table, small P-value: significant result.
- Conclude that the mean bone densities are not all equal.
$\rightarrow$ 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.
$\rightarrow$ 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. Iow

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 eq 95 percent confidence interval:
-33.83725 11.03725
sample estimates:
mean of $x$ mean of $y$
$601.1 \quad 612.5$
No sig. difference here.

## Control vs. high

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

Welch Two Sample t-test
data: controls\$density and highs\$density
$\mathrm{t}=-3.7155$, $\mathrm{df}=14.831$, p -value $=0.002109$
alternative hypothesis: true difference in means is not eq 95 percent confidence interval:
-59.19139-16.00861
sample estimates:
mean of $x$ mean of $y$
$601.1 \quad 638.7$
These are different.

## Low vs. high

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

Welch Two Sample t-test
data: lows\$density and highs\$density
$\mathrm{t}=-3.2523, \mathrm{df}=17.597, \mathrm{p}$-value $=0.004525$
alternative hypothesis: true difference in means is not eq 95 percent confidence interval:

$$
-43.15242 \quad-9.24758
$$

sample estimates:
mean of $x$ mean of $y$
$612.5 \quad 638.7$
These are different too.

## But...

We just did 3 tests instead of 1 .
$\rightarrow$ 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



## 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.
$\rightarrow$ Any means unusually different compared to that declared significantly different.


## Tukey on rat data

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

|  | Df | Sum Sq Mean Sq F value $\operatorname{Pr}(>F)$ |  |  |  |
| :--- | ---: | ---: | ---: | ---: | :--- |
| group | 2 | 7434 | 3717 | 7.978 | 0.0019 | **

Signif. codes:
$0{ }^{\prime} * * * ' 0.001{ }^{\prime} * * ' 0.01 '^{\prime \prime} 0.05 '^{\prime} 0.0 .1 '^{\prime} 1$

## TukeyHSD (rats.aov)

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

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

$$
\text { diff lwr upr } \quad \text { p adj }
$$

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

Look at P-values for the two tests:
Comparison Tukey t-tests

Highjump-Control $0.0016 \quad 0.0021$
Lowjump-Control $0.4744 \quad 0.2977$
Lowjump-Highjump $0.0298 \quad 0.0045$

- 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
$\rightarrow$ 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 |

## 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

| $\quad$ g1 | g2 | p_value | adj_p_value |  |
| :--- | :--- | :--- | ---: | ---: |
|  | <chr> | <chr> | <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.
$\rightarrow$ Appropriate Tukey-equivalent here called Games-Howell.


## Games-Howell

- Lives in package PMCMRplus. Install first.


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

Control Highjump
Highjump 0.0056 -
Lowjump 0.54170 .0120

## Deciding which test to do

For two or more samples:


