

## 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 ( $\text{mg}/\text{cm}^3$ ) 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, " ")
```

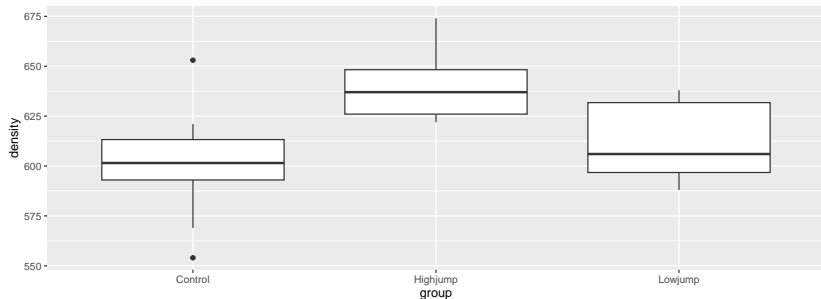
## The data (some random rows)

```
rats %>% slice_sample(n=12)
```

```
# A tibble: 12 x 2
  group      density
  <chr>      <dbl>
1 Highjump    643
2 Lowjump     599
3 Control     611
4 Lowjump     605
5 Lowjump     635
6 Lowjump     588
7 Control     621
8 Lowjump     632
9 Lowjump     596
10 Highjump    650
11 Control     554
12 Lowjump     631
```

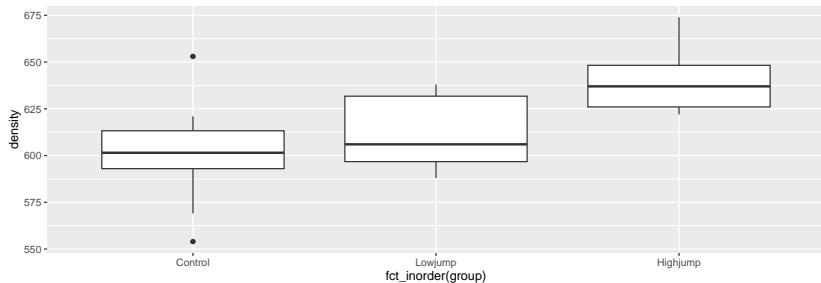
# Boxplots

```
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)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
group	2	7434	3717	7.978	0.0019 **
Residuals	27	12579	466		

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 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 equal to 0

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 equal to 0

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 equal to 0

95 percent confidence interval:

-43.15242 -9.24758

sample estimates:

mean of x mean of y

612.5 638.7

These are different too.

But...

- ▶ 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)
TukeyHSD(rats.aov)
```

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

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

\$group

	diff	lwr	upr	p adj
Highjump-Control	37.6	13.66604	61.533957	0.0016388
Lowjump-Control	11.4	-12.53396	35.333957	0.4744032
Lowjump-Highjump	-26.2	-50.13396	-2.266043	0.0297843

- ▶ Again conclude that bone density for highjump group significantly higher than for other two groups.

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

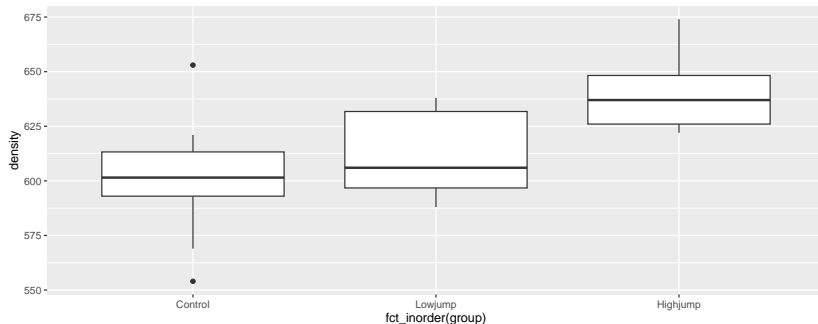
Look at P-values for the two tests:

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()
```

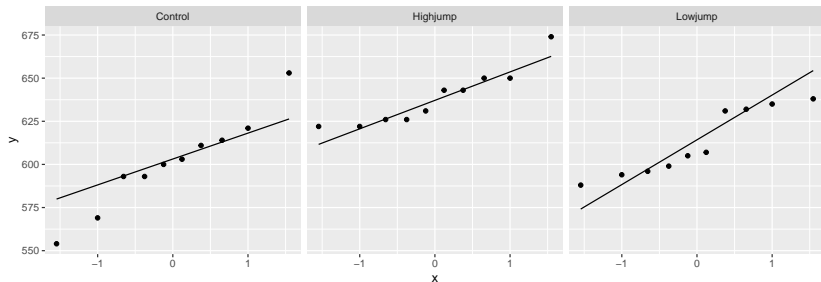


Assumptions:

- ▶ 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
Lowjump	4	6

## 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>	<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.

# 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:

