Master Sciences Pour l'Environnement

Parcours Gestion de l'Environnement et Écologie Littorale

Analyse de données / Data analysis
Partie 4 / Part 4

B. Simon-Bouhet

La Rochelle Université

First semester

What will we talk about?…

10. Comparing two means
    - Paired comparison of means
    - Two-sample comparison of means
    - How to check t-test's assumptions?
    - Nonparametric alternatives to t-tests
    - The fallacy of indirect comparison
    - Interpreting overlap of confidence intervals
11. Comparing means of more than two groups
    - One-factor ANOVA
    - Assumptions and alternatives
    - Post-hoc tests
    - Fixed and random effects
    - Two factors ANOVA
    - Bloc design and nested factors
12. Introduction to linear models
    - Linear regression
    - ANCOVA: analysis of covariance

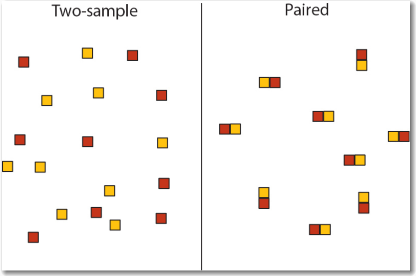
10. Comparing two means

Outline

Paired vs independent designs

Definition

In a paired design, both treatments are applied to every sampled unit.
In a independent design, each treatment group is composed of an independent random sample of units.



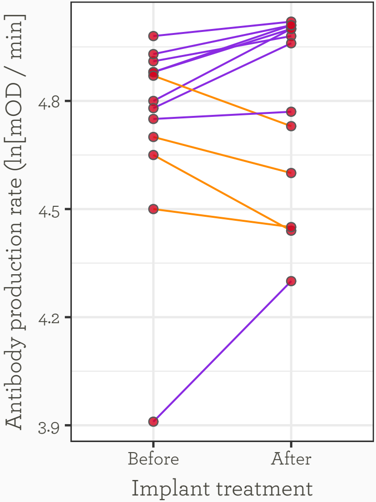
Important

Paired measurements are converted to a single measurement by taking the difference between them.

Estimating the mean difference from paired data

So macho it makes you sick

Data by Hasselquist *et al.* (1999)



Paired t-test

So macho it makes you sick

Male identification number	Antibody production before implant (ln[mOD/min])	Antibody production after implant (ln[mOD/min])	d
1	4.65	4.44	-0.21
2	3.91	4.30	0.39
3	4.91	4.98	0.07
4	4.50	4.45	-0.05
5	4.80	5.00	0.20
6	4.88	5.00	0.12
7	4.88	5.01	0.13
8	4.78	4.96	0.18
9	4.98	5.02	0.04
10	4.87	4.73	-0.14
11	4.75	4.77	0.02
12	4.70	4.60	-0.10
13	4.93	5.01	0.08

Paired t-test

So macho it makes you sick

1. **State the hypothesis**
   - H_0: The mean change in antibody production after testosterone implant is zero, ($\mu_d = 0$)
   - H_A : The mean change in antibody production after testosterone implant is not zero ($\mu_d \neq 0$)

Important

Performing a paired t-test of equality of mean is like performing a one sample t-test on the difference of means with $\mu_0 = 0$.

Paired t-test

So macho it makes you sick

2 & 3. Compute the test statistic and the P-value

Paired t-test

So macho it makes you sick

2 & 3. Compute the test statistic and the P-value

```
blackbird$logAfterImplant
```

```
 [1] 4.44 4.30 4.98 4.45 5.00 5.00 5.01 4.96 5.02 4.73 4.77
[12] 4.60 5.01
```

```
blackbird$logBeforeImplant
```

```
 [1] 4.65 3.91 4.91 4.50 4.80 4.88 4.88 4.78 4.98 4.87 4.75
[12] 4.70 4.93
```

```
d <- blackbird$logAfterImplant - blackbird$logBeforeImplant
d
```

```
 [1] -0.21  0.39  0.07 -0.05  0.20  0.12  0.13  0.18  0.04
[10] -0.14  0.02 -0.10  0.08
```

Paired t-test

So macho it makes you sick

2 & 3. Compute the test statistic and the P-value

```
t.test(blackbird$logAfterImplant, blackbird$logBeforeImplant,
    paired = TRUE)


^^IPaired t-test

data:  blackbird$logAfterImplant and blackbird$logBeforeImplant
t = 1.2714, df = 12, p-value = 0.2277
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.04007695  0.15238464
sample estimates:
mean of the differences
             0.05615385
```

Paired t-test

So macho it makes you sick

2 & 3. Compute the test statistic and the P-value

```
t.test(d, mu = 0)


^^IOne Sample t-test

data:  d
t = 1.2714, df = 12, p-value = 0.2277
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.04007695  0.15238464
sample estimates:
 mean of x
0.05615385
```

Paired t-test

So macho it makes you sick

Important

Assumption of the test:

- The sampling units are randomly sampled from the population.
- The paired differences have a normal distribution in the population.

Outline

Some data to work with

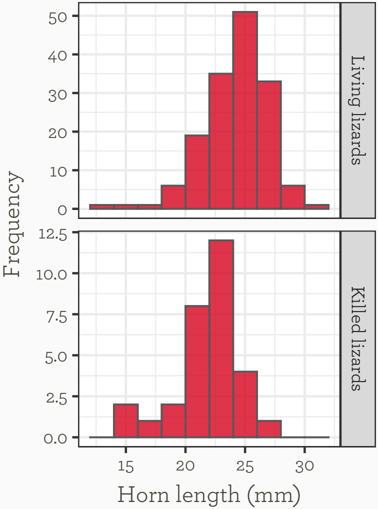
Spike or be spiked

The horned lizard *Phrynosoma mcallii*, data from Young *et al.* (2004).

Lizard group	Sample mean $\overline{Y}$ (mm)	Sample standard deviation s (mm)	Sample size (n)
Living	24.28	2.63	154
Killed	21.99	2.71	30

Frequency distribution of horn length

Spike or be spiked



Both frequency distributions seems to have roughly:

- the shape of a normal distribution.
- a similar spread.

The two-sample t-test

Spike or be spiked

Assumptions of the test

- Each of the two samples is a random sample from its population.
- The numerical variable is normally distributed in each population.
- The standard deviation (and variance) of the numerical variable is the same in both populations.

1. State the hypotheses

- H_0: $\mu_1 = \mu_2$, i.e. $\mu_1 - \mu_2 = 0$
- H_A: $\mu_1 \neq \mu_2$, i.e. $\mu_1 - \mu_2 \neq 0$

The two-sample t-test

Spike or be spiked

2-3. Test statistic and p-value

```
t.test(HornLength ~ Status, data = lizard, var.equal = TRUE)


^^ITwo Sample t-test

data:  HornLength by Status
t = 4.3494, df = 182, p-value = 2.27e-05
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 1.253602 3.335402
sample estimates:
mean in group Living lizards mean in group Killed lizards
                    24.28117                     21.98667
```

The Welch's approximate t-test

Spike or be spiked

Definition

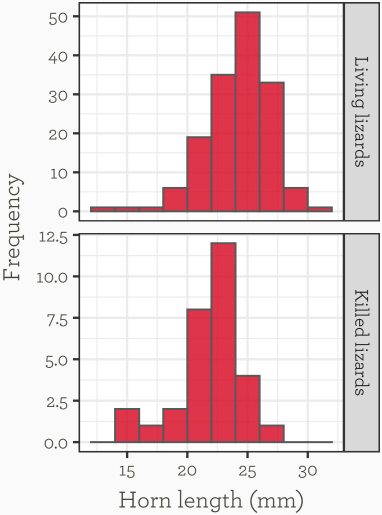
Welch's t-test compares the mean of two groups and can be used even when the variances of the two groups are not equal.

```
t.test(HornLength ~ Status, data = lizard)


^^IWelch Two Sample t-test

data:  HornLength by Status
t = 4.2634, df = 40.372, p-value = 0.0001178
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 1.207092 3.381912
sample estimates:
mean in group Living lizards mean in group Killed lizards
                    24.28117                     21.98667
```

Outline

Checking normality

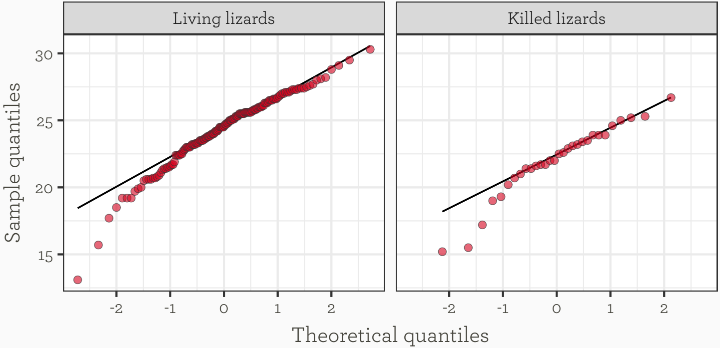
Graphical method: frequency distribution



Checking normality

Graphical method: quantile-quantile plots

```
ggplot(lizard, aes(sample = HornLength)) +
  geom_qq_line() +
  geom_qq(shape = 21, color = "grey20", fill = mycol,
          size = 2, stroke = 0.3, alpha = 0.6) +
  facet_wrap(~ Status) +
  labs(x = "Theoretical quantiles", y = "Sample quantiles")
```



Checking normality

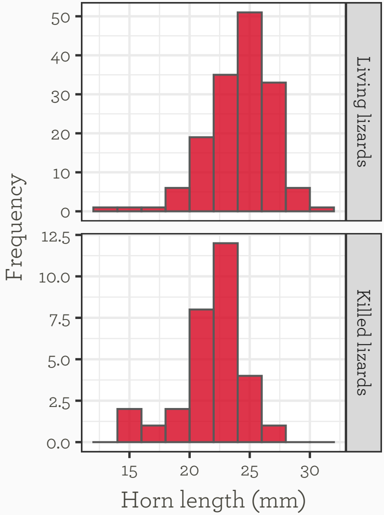
Formal comparison: the Shapiro-Wilk test

1. **State the hypotheses**
   - H_0: The data are sampled form a normal population.
   - H_A: The data are sampled form a non-normal population.

```
# Tidyverse-friendly versions of many statistical tests
library(rstatix)

lizard %>%
  group_by(Status) %>%
  summarise(shapiro_test(HornLength), .groups = "drop")

# A tibble: 2 x 4
  Status         variable   statistic  p.value
  <fct>          <chr>          <dbl>    <dbl>
1 Living lizards HornLength     0.961 0.000223
2 Killed lizards HornLength     0.935 0.0648
```

Checking the homogeneity of variances

Graphical method: frequency distribution



Checking the homogeneity of variances

Formal test 1: the F-test of equal variances

1. State the hypotheses

- H_0: $\sigma_1^2 = \sigma_2^2 \iff \dfrac{\sigma_1^2}{\sigma_2^2} = 1.$
- H_A: $\sigma_1^2 \neq \sigma_2^2 \iff \dfrac{\sigma_1^2}{\sigma_2^2} \neq 1.$

```
var.test(HornLength ~ Status, data = lizard)


^^IF test to compare two variances

data:  HornLength by Status
F = 0.94276, num df = 153, denom df = 29, p-value =
0.7859
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.5042509 1.5774536
sample estimates:
ratio of variances
         0.9427641
```

Checking the homogeneity of variances

Formal test 2: Levene's test for homogeneity of variances

1. **State the hypotheses**
   - H_0: The variance of all groups is the same.
   - H_A: At least one group has a variance that differs from the others.

```
# library(rstatix)
lizard %>%
  levene_test(HornLength ~ Status)
```

```
# A tibble: 1 x 4
    df1   df2 statistic     p
  <int> <int>     <dbl> <dbl>
1     1   182   0.00348 0.953
```

Outline

Definition and principles

Useful but less powerful

> ***Definition***
>
> A nonparametric method makes fewer assumptions than standard parametric methods do about the distribution of the variables.

Nonparametric tests are applicable when parametric tests are not. But they use less information from the data than parametric tests do.

The consequence is a smaller (sometimes, much smaller) power compared to parametric tests.

The Wilcoxon test

One name, various tests

The `wilcox.test()` function can perform 3 different tests:

- `wilcox.test(sample1, mu = value)`: Wilcoxon signed rank test. Nonparametric equivalent of the one-sample t-test.
- `wilcox.test(sample1, sample2, paired = TRUE)`: Wilcoxon signed rank test. Nonparametric equivalent of the two-sample paired t-test.
- `wilcox.test(sample1, sample2, paired = FALSE)`: Wilcoxon rank sum test, aka the Mann-Whitney U-test. Nonparametric equivalent of the two-sample t-test.

For each of these tests, the null and alternative hypotheses are the same as for their parametric equivalent, but the position parameter tested is not necessarily the mean.

Add `conf.int = TRUE` to compute and print estimates and confidence intervals.

The Wilcoxon test

Examples with fake data

```
# Generate random samples
a <- runif(40, min = 10, max = 15)
b <- runif(40, min = 13, max = 20)
a
```

```
 [1] 12.25634 13.91890 13.54841 11.90872 13.18162 13.50673
 [7] 13.20219 11.33340 14.07711 14.91493 10.13634 14.18745
[13] 13.01620 12.83727 14.10026 11.25786 12.52747 14.33769
[19] 14.79091 12.72849 10.69790 14.77670 11.96247 11.34243
[25] 12.86103 14.56072 14.67146 14.40243 14.72847 14.07495
[31] 10.16388 14.71355 14.73869 14.51046 12.76133 11.12443
[37] 14.65357 11.20033 14.67450 12.23667
```

```
mean(a)
```

```
[1] 13.16561
```

```
mean(b)
```

```
[1] 16.10189
```

The Wilcoxon test

Examples with fake data

```
# Normality tests
shapiro.test(a)


^^IShapiro-Wilk normality test

data:  a
W = 0.90764, p-value = 0.003215
```

```
shapiro.test(b)


^^IShapiro-Wilk normality test

data:  b
W = 0.91357, p-value = 0.004861
```

The Wilcoxon test

Examples with fake data

```
# Wilcoxon signed-rank test
wilcox.test(a, mu = 11, conf.int = TRUE)


^^IWilcoxon signed rank exact test

data:  a
V = 801, p-value = 5.584e-10
alternative hypothesis: true location is not equal to 11
95 percent confidence interval:
 12.71830 13.75259
sample estimates:
(pseudo)median
      13.20716
```

The Wilcoxon test

Examples with fake data

```
# Wilcoxon signed-rank test for paired samples
wilcox.test(a, b, paired = TRUE, conf.int = TRUE)


^^IWilcoxon signed rank exact test

data:  a and b
V = 29, p-value = 3.163e-09
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -3.721857 -2.147605
sample estimates:
(pseudo)median
     -2.903023
```

The Wilcoxon test

Examples with fake data

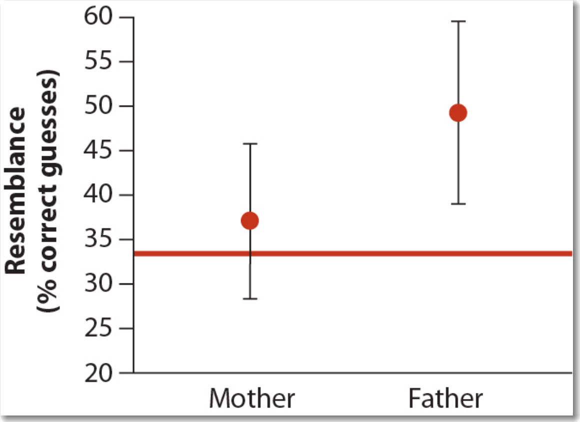
```
# Wilcoxon rank-sum test for independent samples
wilcox.test(a, b, conf.int = TRUE)


^^IWilcoxon rank sum exact test

data:  a and b
W = 260, p-value = 4.104e-08
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -3.898800 -1.889135
sample estimates:
difference in location
             -2.903588
```

Outline

Some data to work with

Mommy's baby, Daddy's maybe

Do babies look more like their mother or father ? Data from Christenfeld & Hill (1995).



The fallacy of indirect comparison

Mommy's baby, Daddy's maybe

The study showed the following:

- The null hypothesis of no resemblance (i.e. one third correct guesses) was soundly rejected for fathers.
- The null hypothesis of no resemblance was not rejected for mothers.

The researchers concluded:

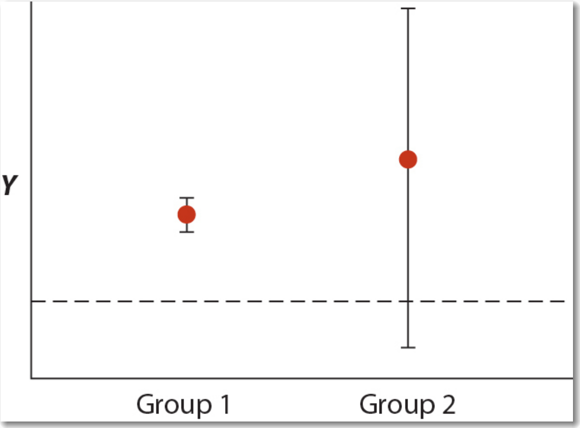
Babies resemble their fathers more than they resemble their mothers.

This is an indirect comparison. This is WRONG!

If mothers and fathers had been compared to one another directly, no significant difference would have been found.

The fallacy of indirect comparison

A more extreme case



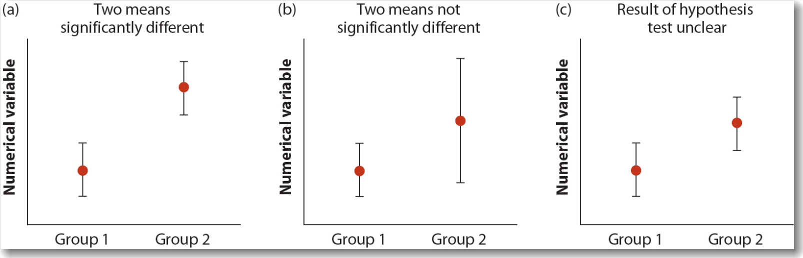
The fallacy of indirect comparison

Comparing 3 means

Outline

Interpreting overlap of confidence intervals

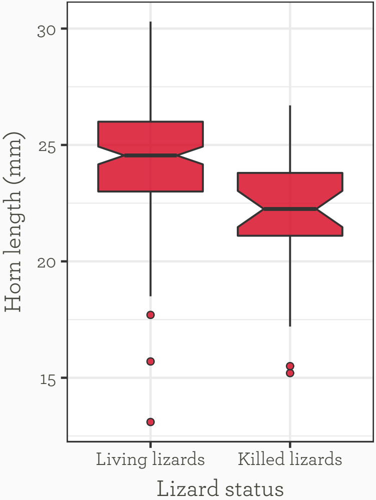
Sometimes, researchers report the means of two or more groups along with their 95% confidence intervals, but do not test the difference between the means with formal statistical tests.
Are confidence intervals enough to draw conclusions about the difference between means?



Reliable conclusions can only be reached in panels (a) and (b).

Interpreting overlap of confidence intervals

Confidence intervals for the median



11. Comparing means of more than two groups

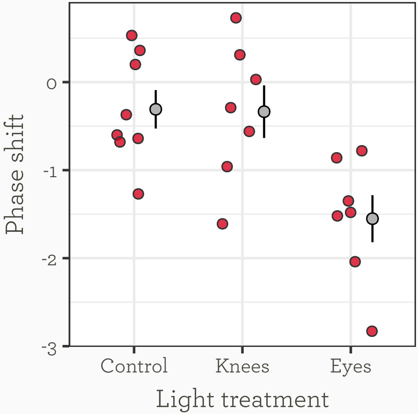
Outline

Some data to work with

Knees and jet lag

Does light treatment affect phase shift? Data from Wright & Czeisler (2002) in response to an earlier study by Campbell & Murphy (1998).

Treatment	$\bar{Y}$	s	n
Control	-0.309	0.618	8
Knees	-0.336	0.791	7
Eyes	-1.551	0.706	7



One-factor ANOVA

Knees and jet lag

Definition

Analysis of variance is the most powerful approach known for simultaneously testing whether the means of k groups are equal. It tests whether individuals from different groups are, on average, more different than individuals chosen from the same group.

1. State the hypotheses

- H_0: $\mu_1 = \mu_2 = \mu_3$.
- H_A: At least one mean is different from the others.

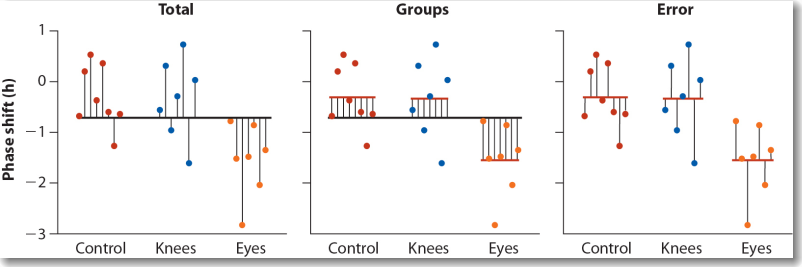
Important

Rejecting H_0 does not mean that all means are different, but that at least one mean is different from the others.

One-factor ANOVA

Knees and jet lag

Source of variation	Sum of squares	*df*	Mean squares	F-ratio	P
Groups (treatment)	7.224	2	3.6122	7.29	0.004
Error	9.415	19	0.4955		
Total	16.639	21			



$$Y_{ij} - \overline{Y} = (Y_{ij} - \overline{Y}_i) + (\overline{Y}_i - \overline{Y})$$

One-factor ANOVA

Knees and jet lag

Here is what it looks like in R:

```
# Perform the ANOVA...
circadianAnova <- aov(shift ~ treatment, data = circadian)

# ... and print the ANOVA table
anova(circadianAnova)
```

```
Analysis of Variance Table

Response: shift
          Df Sum Sq Mean Sq F value   Pr(>F)
treatment  2 7.2245  3.6122  7.2894 0.004472 **
Residuals 19 9.4153  0.4955
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

One-factor ANOVA

Knees and jet lag

Definition

R^2 measures the fraction of the variation in Y that is explained by group differences.

```
circadianAnovaSummary <- summary.lm(circadianAnova)
circadianAnovaSummary$r.squared
```

```
[1] 0.4341684
```

4. Draw the appropriate conclusions

Since $P < \alpha$, we reject H_0. At least one of the groups has a mean that is different from the others.

Outline

Assumptions of the ANOVA

The assumptions are the same as for the t-test, but they must hold for all k groups:

- The measurements in every group represent a random sample from the corresponding population.
- The variable is normally distributed in each of the k populations
- The variance is the same in all k populations

Very important!

In practice, contrary to all other tests described up to this point, the assumptions are verified :

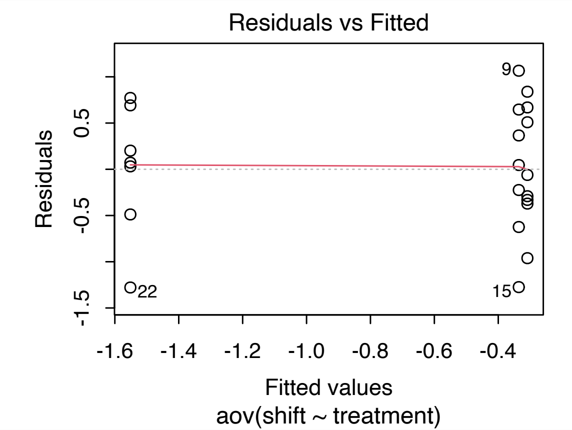
- **after** the analysis is done.
- not on the data themselves, but on the **ANOVA residuals.**

This is true for **all linear models**.

Assumptions of the ANOVA

Checking the residuals

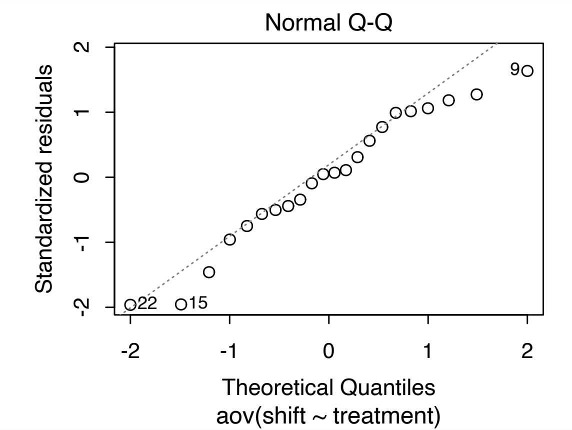
```r
plot(circadianAnova)
```



Assumptions of the ANOVA

Checking the residuals

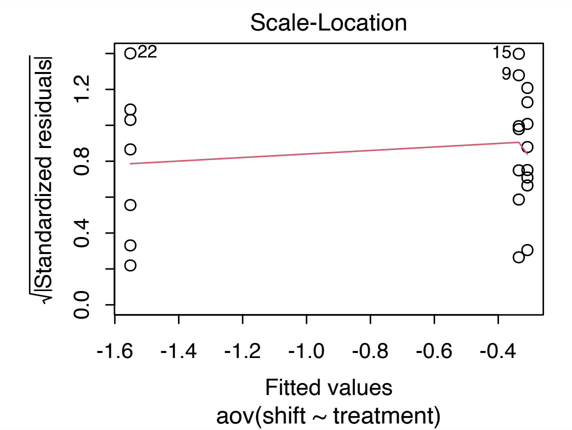
```r
plot(circadianAnova)
```



Assumptions of the ANOVA

Checking the residuals

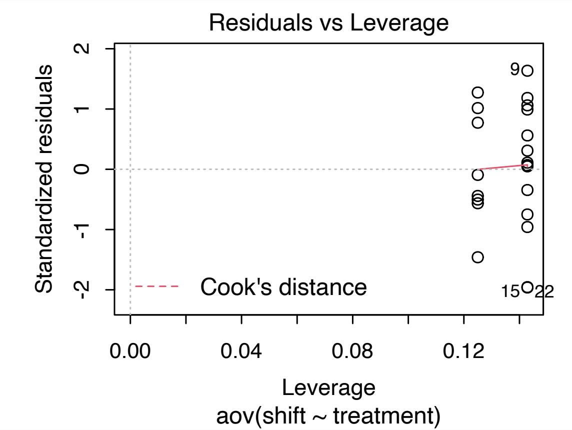
```r
plot(circadianAnova)
```



Assumptions of the ANOVA

Checking the residuals

```r
plot(circadianAnova)
```



What if assumptions are not met?

Nonparametric alternative

Definition

The Kruskal-Wallis test is the nonparametric equivalent to the one-factor ANOVA. It should be used when data (*i.e.* residuals) are not normally distributed.

```
kruskal.test(shift ~ treatment, data = circadian)


^^IKruskal-Wallis rank sum test

data:  shift by treatment
Kruskal-Wallis chi-squared = 9.4231, df = 2, p-value
= 0.008991
```

Outline

We rejected H_0. Now what?

Differences between groups

Definition

Post-hoc tests perform all possible pairwise comparisons of means and apply corrections so that the type I error does not inflate.

We rejected H_0. Now what?

Differences between groups

Definition

Post-hoc tests perform all possible pairwise comparisons of means and apply corrections so that the type I error does not inflate.

```
library(broom)
tukey <- tidy(TukeyHSD(circadianAnova))
tukey
```

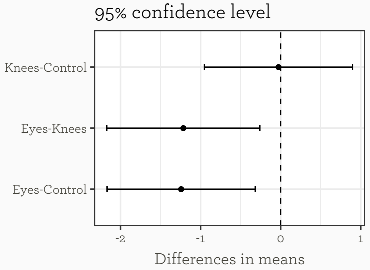
```
# A tibble: 3 x 7
  term      contrast      null.value estimate conf.low conf.high adj.p.value
  <chr>     <chr>              <dbl>    <dbl>    <dbl>     <dbl>       <dbl>
1 treatment Knees-Control          0  -0.0270   -0.953     0.899     0.997
2 treatment Eyes-Control           0  -1.24     -2.17     -0.317     0.00787
3 treatment Eyes-Knees             0  -1.22     -2.17     -0.260     0.0117
```

We rejected H_0. Now what?

Differences between groups

> ***Definition***
>
> Post-hoc tests perform all possible pairwise comparisons of means and apply corrections so that the type I error does not inflate.

```r
tukey %>%
  ggplot(aes(x = estimate,
             y = contrast)) +
  geom_point() +
  geom_errorbarh(aes(xmin = conf.low,
                     xmax = conf.high),
                 height = 0.1) +
  geom_vline(xintercept = 0,
             linetype = 2) +
  labs(x = "Differences in means",
       y = "",
       title = "95% confidence level")
```



We rejected H_0. Now what?

Differences between groups

The pairwise t-test:

```
pairwise.t.test(circadian$shift, g = circadian$treatment)


^^IPairwise comparisons using t tests with pooled SD

data:  circadian$shift and circadian$treatment

      Control Knees
Knees 0.9418  -
Eyes  0.0088  0.0088

P value adjustment method: holm
```

We rejected H_0. Now what?

Differences between groups

The pairwise Wilcoxon test:

```
pairwise.wilcox.test(circadian$shift, g = circadian$treatment)


^^IPairwise comparisons using Wilcoxon rank sum exact test

data:  circadian$shift and circadian$treatment

      Control Knees
Knees 0.9551  -
Eyes  0.0037  0.0524

P value adjustment method: holm
```

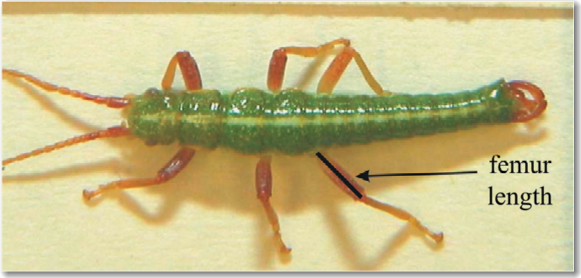
Outline

ANOVA with random effects

Walking-stick limb

Definition

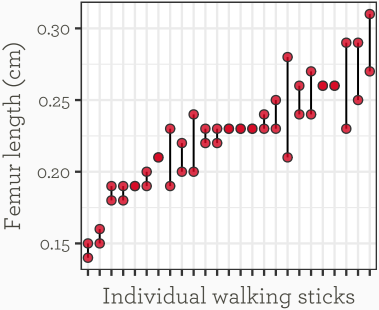
An explanatory variable is called a **fixed effect** if the groups are predetermined and are of direct interest.
An explanatory variable is called a **random effect** if the groups are randomly sampled from a population of possible groups.



Data from Nosil & Crespi (2006).

ANOVA with random effects

Walking-stick limb




```
dim(walkingstick)

[1] 50  2
```

```
walkingstick

# A tibble: 50 x 2
   specimen femurLength
   <fct>          <dbl>
 1 1               0.26
 2 1               0.26
 3 2               0.23
 4 2               0.19
 5 3               0.25
 6 3               0.23
 7 4               0.26
 8 4               0.26
 9 5               0.23
10 5               0.22
# ... with 40 more rows
```

ANOVA with random effects

Walking-stick limb

In R formulae, the `Error()` function is used to indicate random effects.

```
stickaov <- aov(femurLength ~ Error(specimen), data = walkingstick)
summary(stickaov)


Error: specimen
          Df  Sum Sq  Mean Sq F value Pr(>F)
Residuals 24 0.05913 0.002464

Error: Within
          Df Sum Sq  Mean Sq F value Pr(>F)
Residuals 25 0.0089 0.000356
```

Here, we don't have any P-value. Since we only have one variable of interest, our goal is to estimate the variance components: which part of the total variation in femur length is due to measurement errors and which part is due to real differences among individuals?

Random effects: variance components

Walking-stick limb

Definition

In a random effect ANOVA, σ^2 and σ_A^2 are called variance components. They describe all the variance in the response variable Y:

- σ^2 describe the variance within groups (i.e. here, the measurement error).
- σ_A^2 describe the variance among groups (i.e. here, the differences between the true femur lengths of individual insects).

$$s_A^2 = \frac{\mathrm{MS}_{\mathrm{groups}} - \mathrm{MS}_{\mathrm{error}}}{n} = \frac{0.002464 - 0.000356}{2} = 0.00105\,\mathrm{cm}^2$$

where n is the number of measurements taken within each group.

Random effects: variance components

Walking-stick limb

Definition

Repeatability measures the overall similarity of repeat measurements made on the same group.

- When repeatability ≈ 0, all variance in the response variable comes from differences between separate measurements made on the same group.
- When repeatability ≈ 1, repeated measurements on the same group give nearly the same answer every time.

Once we have s_A^2, we can calculate the repeatability:

$$\text{Repeatability} = \frac{s_A^2}{s_A^2 + \text{MS}_{\text{error}}} = \frac{0.00105}{0.00105 + 0.000356} = 0.75$$

Outline

An example with 2 fixed factors

Academic grades, levels and teaching method

Grades of undergrad and grad students depending of the teaching methods they have been exposed to.

	Teach.meth.1	Teach.meth.2
Undergrad	8.00	6.00
	11.00	7.00
	8.00	8.00
	9.00	7.00
Grad	16.00	12.00
	18.00	11.00
	17.00	12.00
	17.00	9.00

An example with 2 fixed factors

Hypotheses sets

Here, we have 3 sets of hypotheses

- H_0: The level of students has no effect on the grades ($\mu_{\text{undergrad}} = \mu_{grad}$).
- H_A: The level of students has an effect on the grades ($\mu_{\text{undergrad}} \neq \mu_{grad}$).

- H_0: The teaching method has no effect on the grades ($\mu_{\text{method 1}} = \mu_{method2}$).
- H_A: The teaching method has an effect on the grades ($\mu_{\text{method 1}} \neq \mu_{method2}$)

- H_0: The teaching methods have the same influence on both groups of students.
- H_A: The teaching methods do not have the same influence on both groups of students.

An example with 2 fixed factors

Data format

```
   Grades Teaching     Level
1       8 Method.1 Undergrad
2      11 Method.1 Undergrad
3       8 Method.1 Undergrad
4       9 Method.1 Undergrad
5      16 Method.1      Grad
6      18 Method.1      Grad
7      17 Method.1      Grad
8      17 Method.1      Grad
9       6 Method.2 Undergrad
10      7 Method.2 Undergrad
11      8 Method.2 Undergrad
12      7 Method.2 Undergrad
13     12 Method.2      Grad
14     11 Method.2      Grad
15     12 Method.2      Grad
16      9 Method.2      Grad
```

An example with 2 fixed factors

The test

```
res <- aov(Grades ~ Teaching * Level, data = dat)
summary(res)

               Df Sum Sq Mean Sq F value   Pr(>F)
Teaching        1     64   64.00      48 1.59e-05 ***
Level           1    144  144.00     108 2.36e-07 ***
Teaching:Level  1     16   16.00      12  0.00468 **
Residuals      12     16    1.33
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The 3 null hypotheses are rejected.

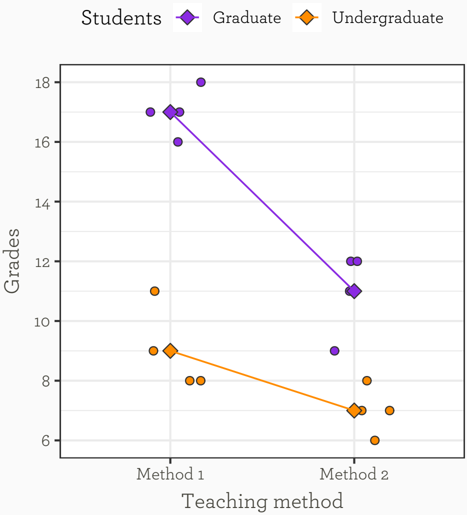
An example with 2 fixed factors

Interaction plot

```
attach(dat)
interaction.plot(Teaching, Level, Grades)
```

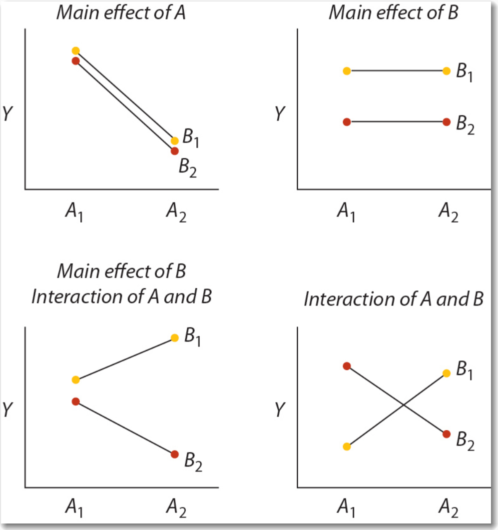
An example with 2 fixed factors

Interaction plot



An example with 2 fixed factors

Interaction plots



ANOVA and formulae

In R, the formulae syntax is not always very intuitive…

We saw that "`Error()`" is used to specify random effects.

The ":" operator is used to indicate an interaction term.

The "*" argument is used to specify a model with both the main effects and the interaction term.

The following commands specify the same model:

```
aov(Grades ~ Teaching * Level, data = dat)
aov(Grades ~ Teaching + Level + Teaching:Level, data = dat)
```

Two factors ANOVA: mixed effects

Zooplankton depredation

Data from Svanbäck & Bolnick (2007).

Treatment	1	2	3	4	5
control	4.1	3.2	3.0	2.3	2.5
low	2.2	2.4	1.5	1.3	2.6
high	1.3	2.0	1.0	1.0	1.6

Treatment	Diversity	Block
control	4.1	1
low	2.2	1
high	1.3	1
control	3.2	2
low	2.4	2
high	2.0	2
control	3.0	3
low	1.5	3
high	1.0	3
control	2.3	4
low	1.3	4
high	1.0	4
control	2.5	5
low	2.6	5
high	1.6	5

Two factors ANOVA: mixed effects

ANOVA the wrong way

```
model1 <- aov(diversity ~ treatment * block, data = zoopk)
summary(model1)
```

```
                Df Sum Sq Mean Sq
treatment        2  6.857   3.429
block            4  2.340   0.585
treatment:block  8  1.676   0.209
```

Two factors ANOVA: mixed effects

ANOVA the right way

```r
model2 <- aov(diversity ~ treatment + Error(block), data = zoopk)
summary(model2)


Error: block
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals  4   2.34   0.585

Error: Within
          Df Sum Sq Mean Sq F value  Pr(>F)
treatment  2  6.857   3.429   16.37 0.00149 **
Residuals  8  1.676   0.209
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Two factors ANOVA: mixed effects

Nonparametric alternative

For this specific design, i.e. two factors ANOVA with mixed effects, when:

- Normality is not met
- Data are not replicated

Two nonparametric tests can be used instead of the ANOVA.

- The Friedman rank sum test with unreplicated blocked data: `friedman.test()`.
- The Quade test with unreplicated blocked data: `quade.test()`.

- The Quade test is more powerful than the Friedman test when there are few treatments (3-4).
- Both tests are equally powerful when there are 5 or 6 treatments
- The Friedman test is more powerful than the Quade test when there are more than 6 treatments.

Two factors ANOVA: mixed effects

The assumption of sphericity

	Block				
Treatment	1	2	3	4	5
control	4.1	3.2	3.0	2.3	2.5
low	2.2	2.4	1.5	1.3	2.6
high	1.3	2.0	1.0	1.0	1.6

Two factors ANOVAs with mixed effects can be considered as one factor ANOVAs with repeated measurements.

Here, each `Block` provides one measurement for each level of the only variable of interest (`treatment`).

Two factors ANOVA: mixed effects

The assumption of sphericity

	Treatment			Differences		
Block	control	low	high	ctrl − low	ctrl − high	low − high
1	4.1	2.2	1.3	1.9	2.8	0.9
2	3.2	2.4	2.0	0.8	1.2	0.4
3	3.0	1.5	1.0	1.5	2.0	0.5
4	2.3	1.3	1.0	1.0	1.3	0.3
5	2.5	2.6	1.6	-0.1	0.9	1.0
			Variance	0.577	0.583	0.097

Definition

Sphericity is met when the differences between all possible pairs of within-subject conditions have equal variance. This is a **very important** condition of repeated-measures ANOVA.

Two factors ANOVA: mixed effects

The assumption of sphericity

The most frequently used test to verify the assumption of sphericity is Mauchly's test (`mauchlys.test`).

> *Important*
>
> Mauchly's test of sphericity should be avoided most of the times !

In practice, we multiply df by $\dfrac{1}{k-1}$, where k is the number of repeated measures (here, 3).

Hence:

```
1 - pf(16.37, df1 = 2 * 0.5, df2 = 8 * 0.5)
```

```
[1] 0.01552707
```

Two factors ANOVA: repeated measures

Actually, three factors with mixed effects

Here, each subject (random factor) provides a measurement for each combination of the two fixed factors of interest.

	Bg low		Bg high	
Subjects	Stim low	Stim high	Stim low	Stim high
S1	12	18	20	8
S2	9	20	24	10
S3	9	22	16	9
S4	10	22	18	11
S5	9	17	18	10
S6	11	23	22	12

Repeated measures ANOVA is way more powerfull than independent ANOVA because one source of variation (the subject error) can be isolated from the main and interaction effects.

Two factors ANOVA: repeated measures

Hypotheses

Here, we have 3 sets of hypotheses:

- H_0: background noise has no effect on the ability to detect the stimuli.
- H_A: background noise has an effect on the ability to detect the stimuli.

- H_0: the frequency of the stimulus has no effect on its detectability.
- H_A: the frequency of the stimulus has an effect on its detectability.

- H_0: the two types of background noises have the same effect on the detectability of the stimuli.
- H_A: the two types of background noises do not have the same effect on the detectability of the stimuli.

Two factors ANOVA: repeated measures

Data format

Data must be presented in the “long format”.

	Score	Noise	Stimulus	Subject
1	12	Low	Low	Subject.1
2	9	Low	Low	Subject.2
3	9	Low	Low	Subject.3
4	10	Low	Low	Subject.4
5	9	Low	Low	Subject.5
6	11	Low	Low	Subject.6
7	18	Low	High	Subject.1
8	20	Low	High	Subject.2
9	22	Low	High	Subject.3
10	22	Low	High	Subject.4
11	17	Low	High	Subject.5
12	23	Low	High	Subject.6

	Score	Noise	Stimulus	Subject
13	20	High	Low	Subject.1
14	24	High	Low	Subject.2
15	16	High	Low	Subject.3
16	18	High	Low	Subject.4
17	18	High	Low	Subject.5
18	22	High	Low	Subject.6
19	8	High	High	Subject.1
20	10	High	High	Subject.2
21	9	High	High	Subject.3
22	11	High	High	Subject.4
23	10	High	High	Subject.5
24	12	High	High	Subject.6

Two factors ANOVA: repeated measures

The test

```
model <- aov(Score ~ Noise * Stimulus
    + Error(Subject / (Noise * Stimulus)), data = hearing)
summary(model)
```

Two factors ANOVA: repeated measures

```
Error: Subject
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals  5   32.5     6.5

Error: Subject:Noise
          Df Sum Sq Mean Sq F value Pr(>F)
Noise      1  0.667   0.667   0.177  0.691
Residuals  5 18.833   3.767

Error: Subject:Stimulus
          Df Sum Sq Mean Sq F value Pr(>F)
Stimulus   1  0.667   0.667   0.124  0.739
Residuals  5 26.833   5.367

Error: Subject:Noise:Stimulus
               Df Sum Sq Mean Sq F value   Pr(>F)
Noise:Stimulus  1  600.0   600.0     240 2.04e-05 ***
Residuals       5   12.5     2.5
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Two factors ANOVA: repeated measures

Conclusions

- H_0: background noise has no effect on the ability to detect the stimuli.
- H_A: background noise has an effect on the ability to detect the stimuli.

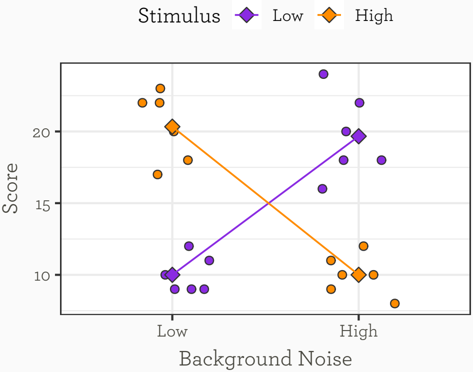
- H_0: the frequency of the stimulus has no effect on its detectability.
- H_A: the frequency of the stimulus has an effect on its detectability.

- H_0: the two types of background noises have the same effect on the detectability of the stimuli.
- H_A: the two types of background noises do not have the same effect on the detectability of the stimuli.

Two factors ANOVA: repeated measures

Conclusions

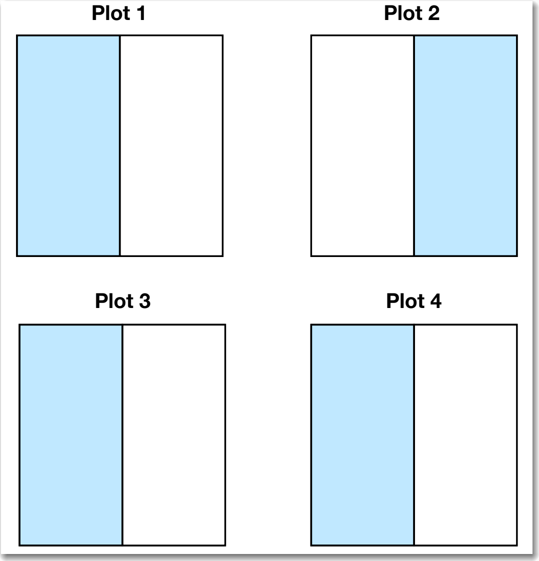
- We have no evidence that background noise has an effect.
- We have no evidence that the frequency of the stimuli has an effect.
- We have evidence ($P < 0.01$) that the two types of background noises do not have the same effect on the detectability of the stimuli.



Outline

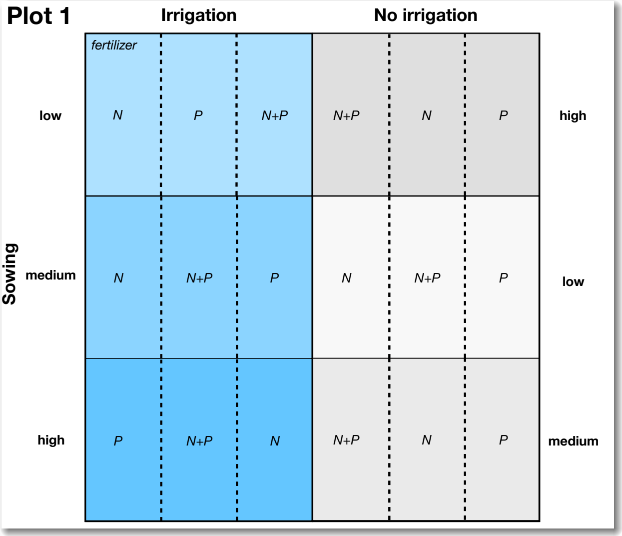
ANOVA 3 nested factors

Experimental design



ANOVA 3 nested factors

Experimental design



ANOVA 3 nested factors

Data format

```
yields <- read_delim("data/splityield.txt", "\t",
                    escape_double = FALSE, trim_ws = TRUE) %>%
          mutate_if(is.character, as_factor)
yields

# A tibble: 72 x 5
   yield block irrigation density fertilizer
   <dbl> <fct> <fct>      <fct>   <fct>
 1    90 A     control    low     N
 2    95 A     control    low     P
 3   107 A     control    low     NP
 4    92 A     control    medium  N
 5    89 A     control    medium  P
 6    92 A     control    medium  NP
 7    81 A     control    high    N
 8    92 A     control    high    P
 9    93 A     control    high    NP
10    80 A     irrigated  low     N
# ... with 62 more rows
```

ANOVA 3 nested factors

Data format

```r
model <- aov(yield ~ irrigation * density * fertilizer +
             Error(block / irrigation / density / fertilizer),
             data = yields)
summary(model)
```

ANOVA 3 nested factors

Data format

```
Error: block
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals  3  194.4   64.81

Error: block:irrigation
           Df Sum Sq Mean Sq F value Pr(>F)
irrigation  1   8278    8278   17.59 0.0247 *
Residuals   3   1412     471
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Error: block:irrigation:density
                   Df Sum Sq Mean Sq F value Pr(>F)
density             2   1758   879.2   3.784 0.0532 .
irrigation:density  2   2747  1373.5   5.912 0.0163 *
Residuals          12   2788   232.3
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

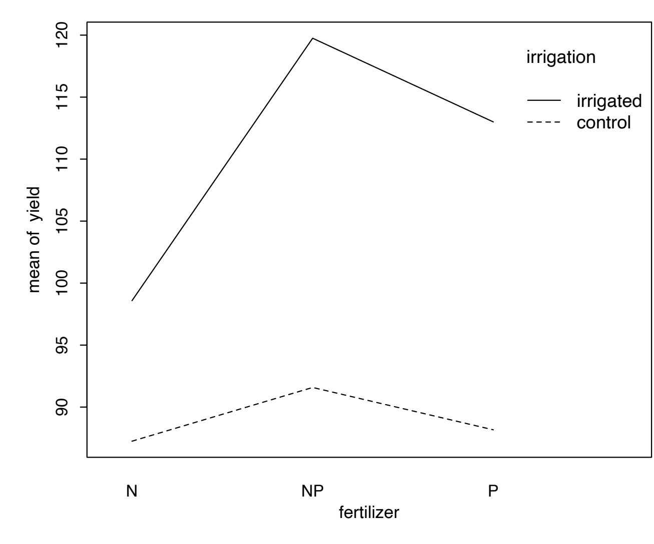
ANOVA 3 nested factors

Data format

```
Error: Within
                              Df Sum Sq Mean Sq F value   Pr(>F)    
fertilizer                     2 1977.4   988.7  11.449 0.000142 ***
irrigation:fertilizer          2  953.4   476.7   5.520 0.008108 ** 
density:fertilizer             4  304.9    76.2   0.883 0.484053    
irrigation:density:fertilizer  4  234.7    58.7   0.680 0.610667    
Residuals                     36 3108.8    86.4                     
---
Signif. codes:  
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

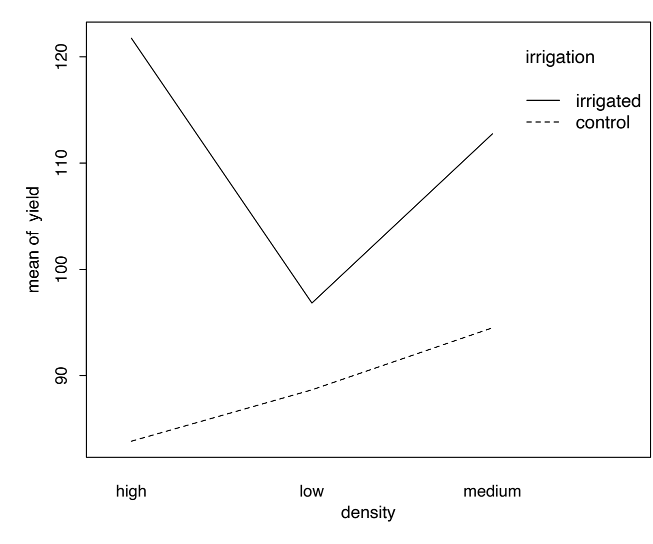
ANOVA 3 nested factors

Understanding significant interactions



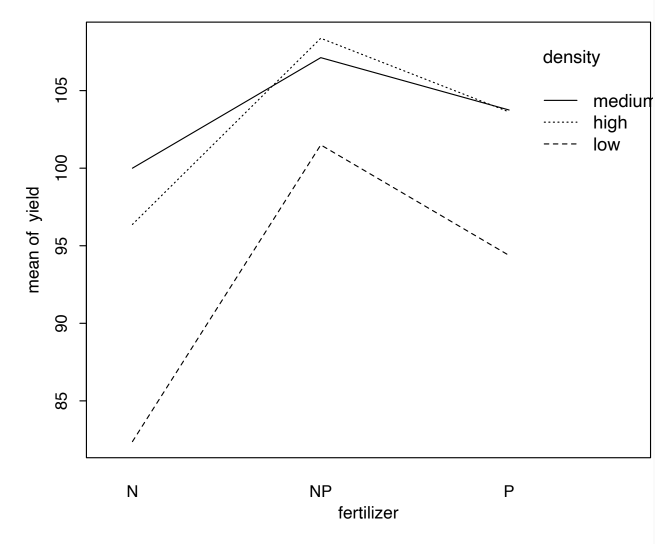
ANOVA 3 nested factors

Understanding significant interactions



ANOVA 3 nested factors

Understanding significant interactions



12. Introduction to linear models

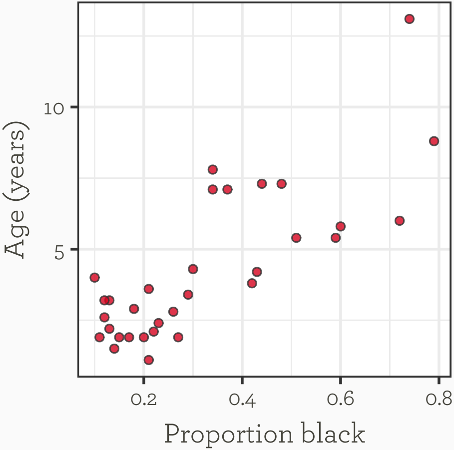
Outline

Simple linear regression

The lion's noses

Data from Whitman *et al.* (2004).

```
# A tibble: 32 x 2
   Prop.black   Age
        <dbl> <dbl>
 1       0.21   1.1
 2       0.14   1.5
 3       0.11   1.9
 4       0.13   2.2
 5       0.12   2.6
 6       0.13   3.2
 7       0.12   3.2
 8       0.18   2.9
 9       0.23   2.4
10       0.22   2.1
# ... with 22 more rows
```



Simple linear regression

Specifying the model

```
lionReg <- lm(Age ~ Prop.black, data = lion)
lionReg


Call:
lm(formula = Age ~ Prop.black, data = lion)

Coefficients:
(Intercept)   Prop.black
      0.879       10.647

confint(lionReg)

                 2.5 %    97.5 %
(Intercept) -0.2826733  2.040686
Prop.black   7.5643082 13.729931
```

Simple linear regression

Testing the slope and intercept

Here, we have 2 sets of hypotheses:

- H_0: the intercept of the linear regression is equal to zero.
- H_A: the intercept of the linear regression is different from zero.

- H_0: the slope of the linear regression is equal to zero.
- H_A: the slope of the linear regression is different from zero.

Confidence intervals gave us a first clue but we can test these hypotheses with the `lm()` function.

Simple linear regression

Testing the slope and intercept

```
summary(lionReg)


Call:
lm(formula = Age ~ Prop.black, data = lion)

Residuals:
    Min      1Q  Median      3Q     Max
-2.5449 -1.1117 -0.5285  0.9635  4.3421

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   0.8790     0.5688   1.545    0.133
Prop.black   10.6471     1.5095   7.053 7.68e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.669 on 30 degrees of freedom
Multiple R-squared:  0.6238,^^IAdjusted R-squared:  0.6113
F-statistic: 49.75 on 1 and 30 DF,  p-value: 7.677e-08
```

Simple linear regression

Confidence and prediction bands

```
# Create a vector of regularly spaced values
x <- seq(from = min(lion$Prop.black),
         to = max(lion$Prop.black),
         length.out = 100)

# Calculate confidence interval
conf <- as_tibble(predict(object = lionReg,
                          newdata = data.frame(Prop.black = x),
                          interval = "confidence"))
names(conf) <- paste0("ci_", names(conf))

# Calculate prediction interval
pred <- as_tibble(predict(object = lionReg,
                          newdata = data.frame(Prop.black = x),
                          interval = "prediction"))
names(pred) <- paste0("pr_", names(pred))

# Merge in a unique tibble
bands <- bind_cols(tibble(x), conf, pred)
```

Simple linear regression

Confidence and prediction bands

```
bands

# A tibble: 100 x 7
        x ci_fit ci_lwr ci_upr pr_fit pr_lwr pr_upr
    <dbl>  <dbl>  <dbl>  <dbl>  <dbl>  <dbl>  <dbl>
 1 0.1      1.94   1.03   2.86   1.94 -1.58    5.47
 2 0.107    2.02   1.12   2.91   2.02 -1.51    5.54
 3 0.114    2.09   1.21   2.97   2.09 -1.43    5.61
 4 0.121    2.17   1.30   3.03   2.17 -1.35    5.68
 5 0.128    2.24   1.39   3.09   2.24 -1.27    5.75
 6 0.135    2.31   1.48   3.15   2.31 -1.19    5.82
 7 0.142    2.39   1.57   3.21   2.39 -1.12    5.89
 8 0.149    2.46   1.66   3.27   2.46 -1.04    5.97
 9 0.156    2.54   1.75   3.33   2.54 -0.961   6.04
10 0.163    2.61   1.83   3.39   2.61 -0.884   6.11
# ... with 90 more rows
```

Simple linear regression

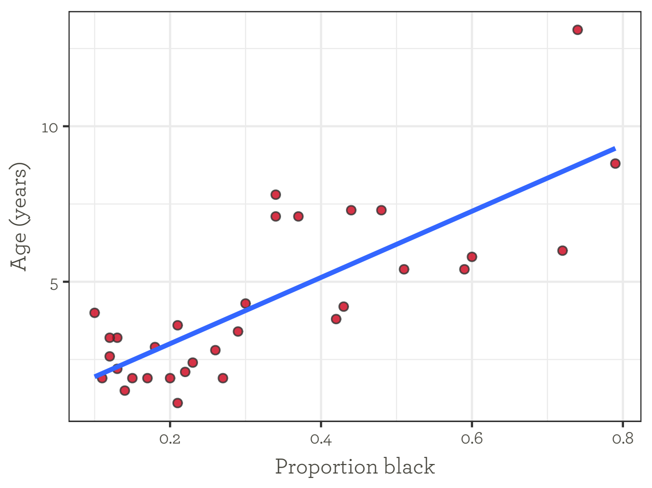
Plotting confidence and prediction bands

```
# Create a scatter plot and add a regression line
pl <- lion %>%
  ggplot(aes(x = Prop.black, y = Age)) +
    geom_point(shape = 21,
               color = grey(0.2),
               fill = rgb(0.84, 0.01, 0.12, 0.8),
               alpha = 0.8) +
    geom_smooth(method = "lm", se = FALSE) +
    labs(x = "Proportion black",
         y = "Age (years)")
```

Simple linear regression

Plotting confidence and prediction bands

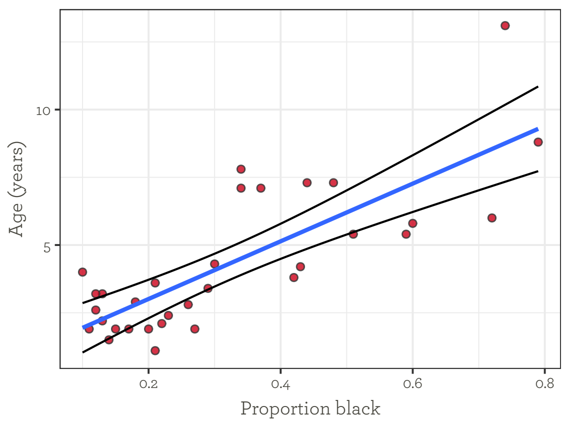
```
pl
```



Simple linear regression

Plotting confidence and prediction bands

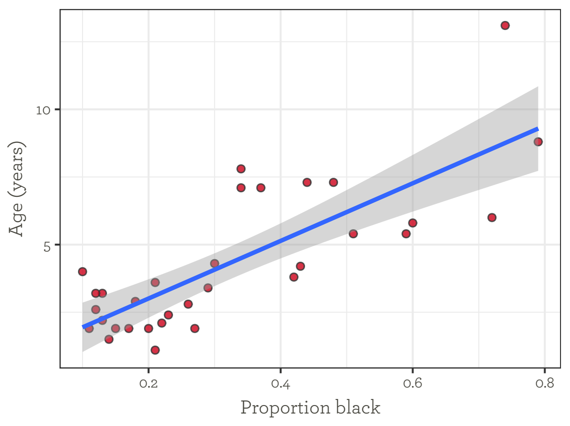
```r
# Add confidence interval
pl + geom_line(data = bands, aes(x = x, y = ci_lwr)) +
     geom_line(data = bands, aes(x = x, y = ci_upr))
```



Simple linear regression

Plotting confidence and prediction bands

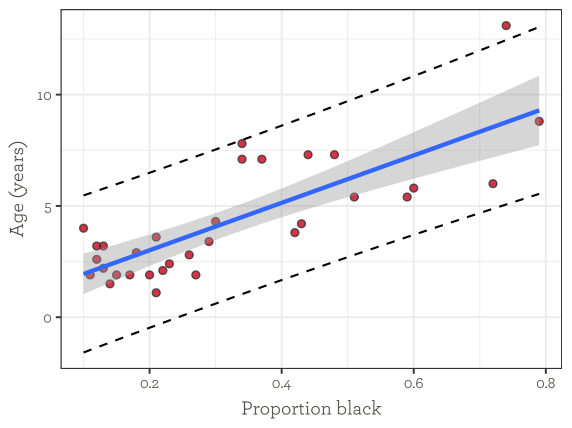
```
# Add confidence interval. Equivalent to :
pl + geom_smooth(method = "lm", se = TRUE)
```



Simple linear regression

Plotting confidence and prediction bands

```r
# Add prediction interval.
pl + geom_smooth(method = "lm", se = TRUE) +
     geom_line(data = bands, aes(x = x, y = pr_lwr), linetype = 2) +
     geom_line(data = bands, aes(x = x, y = pr_upr), linetype = 2)
```



Outline

Mixing regression and ANOVA

Definition and example data

> ***Definition***
>
> The Analysis of Covariance examines the influence of at least two explanatory variables on a single numerical response variable. The explanatory variables are a mix of continuous and categorical factors.

In 2011, Caurant *et al.* studied the relationship between the bioaccumulation of cadmium in the kidney of 244 common dolphins (*Delphinus delphis*) of various ages, coming from 5 distinct geographic regions.

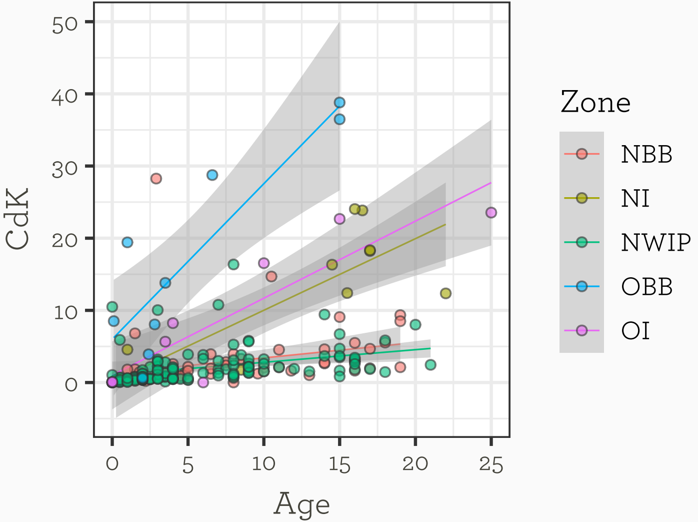
Mixing regression and ANOVA

Definition and example data

```
dauphin

# A tibble: 244 x 3
   Zone    Age   CdK
   <chr> <dbl> <dbl>
 1 NBB    10.5 14.7
 2 NBB    14    2.64
 3 NBB     4.8  1.34
 4 NBB     0.2  0.31
 5 NBB     3    1.66
 6 NBB     1.5  1.83
 7 NBB     7.5  2.83
 8 NBB     1.8  1.43
 9 NBB    17    1.98
10 NBB     2.2  0.22
# ... with 234 more rows
```

Mixing regression and ANOVA

Definition and example data



Mixing regression and ANOVA

Understanding the results

```
# Performing the ANCOVA
ancov <- lm(CdK ~ Zone * Age, data = dauphin)

# Global results
anova(ancov)

Analysis of Variance Table

Response: CdK
           Df Sum Sq Mean Sq F value    Pr(>F)
Zone        4 2869.7  717.43  59.936 < 2.2e-16 ***
Age         1 1237.3 1237.29 103.367 < 2.2e-16 ***
Zone:Age    4 1569.7  392.43  32.785 < 2.2e-16 ***
Residuals 207 2477.8   11.97
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Mixing regression and ANOVA

Understanding the results

```
summary(ancov)$coefficients

                Estimate Std. Error    t value     Pr(>|t|)
(Intercept)   1.28813839 0.68752959  1.8735752 6.239819e-02
ZoneNI       -1.20125790 1.75501252 -0.6844726 4.944424e-01
ZoneNWIP     -0.23286289 0.84014649 -0.2771694 7.819266e-01
ZoneOBB       4.71584521 1.76444627  2.6727055 8.123839e-03
ZoneOI       -0.28732736 1.68968591 -0.1700478 8.651386e-01
Age           0.21222491 0.07696870  2.7572886 6.349305e-03
ZoneNI:Age    0.78025773 0.14784391  5.2775777 3.293708e-07
ZoneNWIP:Age -0.03738055 0.09726805 -0.3843045 7.011473e-01
ZoneOBB:Age   1.94271615 0.22636524  8.5822193 2.215078e-15
ZoneOI:Age    0.85589636 0.16451143  5.2026558 4.713221e-07
```

Mixing regression and ANOVA

Understanding the results

```
# Filter out NBB
dauphin2 <- dauphin %>%
    filter(Zone != "NBB")

# Perform new ANCOVA
ancov2 <- lm(CdK ~ Zone * Age, data = dauphin2)

# Print the results
summary(ancov2)$coefficients

                Estimate Std. Error     t value     Pr(>|t|)
(Intercept)   0.08688049  1.4826028  0.05859998 9.533509e-01
ZoneNWIP      0.96839501  1.5474703  0.62579229 5.324282e-01
ZoneOBB       5.91710311  2.1033812  2.81313874 5.582104e-03
ZoneOI        0.91393054  2.0509786  0.44560706 6.565412e-01
Age           0.99248264  0.1158993  8.56331803 1.408667e-14
ZoneNWIP:Age -0.81763828  0.1281186 -6.38188470 2.183300e-09
ZoneOBB:Age   1.16245842  0.2272369  5.11562423 9.681897e-07
ZoneOI:Age    0.07563863  0.1767889  0.42784719 6.693928e-01
```

References

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