Experimental Design and Data Analysis, Lecture

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Lecture overview

- Analysis of Variance (one-way ANOVA)
- Kruskal-Wallis test
- Open permutation tests in the setting of one-way ANOVA

one way ANOVA (analysis of variance) completely randomized design

to be num., should be

An experiment with:

> we observe smth = response var.
categoric. predictors = factors

- a numerical outcome Y;
- a factor that can be fixed at I levels ("treatment").

If I = 2, this is just the two-sample problem, and we could perform a t-test.

EXAMPLE Agricultural experiment with outcome total yield from a plot and treatment type of fertilizer. Which type of fert, is best ? is there diff, b.w. fort. ?

EXAMPLE Quality of a genetic algorithm to determine the minimal value of a criterion function with outcome CPU time needed to find true minimum and treatment mutation probability set to 0.01, 0.02, 0.03, 0.04 or 0.05.

EXAMPLE Outcome time to develop mold on bread and treatment temperature of the environment fixed to 15, 19 or 22 degrees (garage, bedroom, living room).

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Design

- Select NI experimental units randomly from the population of interest.
- Assign level i of the factor to a random set of N units (i = 1, 2, ..., I).
- Perform the experiment NI times, independently.

```
Randomization in R.

> I=4; N=5
> rep(1:I,N)

[1] 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
> sample(rep(1:I,N))

[1] 3 4 2 1 1 4 3 4 3 1 3 2 3 2 1 4 2 4 2 1

Use level 3 for unit 1, level 4 for unit 2, etc.
```

Using an equal number of units N for each level (called balanced design) is preferable, but not necessary.

One-way ANOVA

```
Data sample 1: Y_{11}, Y_{12}, ..., Y_{1N} sample 2: Y_{21}, Y_{22}, ..., Y_{2N} ... \neq 4 of the obs w/h sample sample 1: Y_{I1}, Y_{I2}, ..., Y_{IN}.
```

```
Samples are indep. b.w. and within samples
```

Assume that these samples are obtained independently from I normal populations with (possibly different) population means $\mu_1, \mu_2, \dots, \mu_I$, and with equal variances.

We want to test the null hypothesis $H_A: \mu_1 = \mu_2 = \cdots = \mu_I$ versus the alternative $H_1: \mu_i \neq \mu_j$ for some (i,j).

The test statistic is a bit complicated, see below. It is, together with its distribution under H_A , implemented in R.

```
We won't to test if sat least 2 abs. should

there is effect of factor have diff. means = we

say treat has effect, if all are the same = treat has no effect
```

One-way ANOVA model

A categorical explanatory variable (also called factor) with I different categories/levels corresponds to I groups/populations/levels.

model =

7 / 32

The one-way ANOVA model is: with $\mu_i = \mu + \alpha_i$, instead of T parameters we now have I+1 params

$$Y_{ik} = \mu_i + e_{ik} = \mu + \alpha_i + e_{ik}, \quad i = 1, \dots, I, \quad k = 1, \dots, n_i,$$

- Y_{ik} is the k-th response measured in group i.
- \Rightarrow in sample, we can have diff. el $i,\ i=1,\ldots,l$ obs. • μ is the common mean, α_i is the contribution of level $i, i = 1, \dots, I$,

Assumption: the indep. errors $e_{ik} \sim N(0, \sigma^2)$, with unknown variance σ^2 .

Balanced design: the same number of observations per group $n_i = N$,

 $i=1,\ldots,I$, so that the total number of observations is $n=\sum_{i=1}^{I}n_i=NI$.

Note: if I = 2, this is the setting for the two sample t-test with equal variances.

> each μ can be expr. by diff. comb.s of $\mu+\infty$ (identif. diff.)

Parameters $\mu, \alpha_1, \dots, \alpha_n$ are not uniquely defined, one needs to specify one linear, restriction on the parameters. Default parametrization (treatment parametrization) in R is $\alpha_1 = 0$, meaning that group 1 is the reference class. Other common

parametrizations are $\mu=0$ (then $\mu_i=\alpha_i$) or $\sum_{i=1}^I \alpha_i=0$. The parametrization in R can be set by the command contrasts.

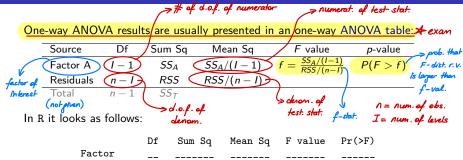
The one-way ANOVA can be written in the matrix form $Y = X\beta + e$ for appropriate design matrix X and parameter vector β , depending on a chosen parametrization.

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One-way ANOVA test

```
Setting: a one-way ANOVA model: Y_{ij} = \mu + \alpha_i + e_{ij}. Treatment doesn't matter
      Hypotheses: H_A: \alpha_1 = \ldots = \alpha_k = 0 (no factor effect) versus H_1: at least one
      \alpha_i \neq 0 (factor effect is present).
      Test statistic: with \bar{Y}_{i.} = \frac{1}{n_i} \sum_{k=1}^{n_i} Y_{ik} and \bar{Y}_{..} = \frac{1}{l} \sum_{i=1}^{l} \frac{1}{n_i} \sum_{k=1}^{n_i} Y_{ik}, under H_0,
                     \frac{\text{between-groups SS}}{\text{within-groups SS}} = \frac{\sum_{i=1}^{I} n_i (\bar{Y}_{i.} - \bar{Y}_{..})^2 / (I-1)}{\sum_{i=1}^{I} \sum_{i=1}^{n_i} (Y_{ij} - \bar{Y}_{i.})^2 / (n-I)} \sim F_{I-1,n-I},
b.w. proups
      the F-distribution with I-1 and n-I degrees of freedom.
      Larger values of F = f give more evidence against H_0 in favor of H_1, hence we
      only reject H_A if F is large. The test is therefore always right-sided: compare
      the p-value p_{right} = P(F > f) with a significance level \alpha.
                                                                                                    ve are anly interested
                                                                                                        In testing whether
      In R: the p-value is in anova (lm(y \sim f), data = ...), f is the factor.
                                                  Im = creates model, y = response
       In R: summary(\lim (y \sim f, data = ...)) shows the coefficient estimates \hat{\alpha}_i's in the
       treatment parameterization, to get these in the sum parametrization use (before 1m
       command) contrasts(f)=contr.sum.
                                                              \hat{\omega}_{:} = fit. value of param.s
```

One-way ANOVA table



Residuals

Here
$$RSS = \sum_{i=1}^{l} \sum_{j=1}^{n_i} (Y_{ij} - \bar{Y}_{i\cdot})^2$$
, $SS_A = \sum_{i=1}^{l} n_i (\bar{Y}_{i\cdot} - \bar{Y}_{\cdot\cdot})^2$, $SS_T = RSS + SS_A$.

The denominator $\frac{RSS}{R}$ of the test statistics F is an unbiased estimator of σ^2 :

$$\hat{\sigma}^2 = S^2 = \frac{RSS}{n-1}.$$

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One-way ANOVA in R: graphics

```
Lile contoins headers
                                                          of col.s -> set to true
  melon=read.table("melon.txt",header=TRUE)
> melon
                                                    adiff. fatilizers — we want to know whether vortely as a fac. plays a role
  variety1 variety2 variety3 variety4
      15.09
                  17.12
                             21.20
                                         18.93
      20.21
                  19.17
                             28.83
                                         31.34
      30.35
                 28.99
                             31.43
                                         30.13
                                                   yields
      25.03
                 22.84
                             25.90
                                         23.18
                  31.72
5
      20.50
                             21.98
                                         19.86
6
      21.50
                  18.67
                             24.48
                                         23.75
  boxplot(melon); stripchart(melon,vertical=TRUE)
to see a
 significant
                                                    25
  not so
  clear +
  Small
  d.set
                        2
                              3
                                                              variety2
                                                                       variety3
                                                                               variety4
```

variety1

One-way ANOVA in R: data input

If needed, create a data frame with a numeric column of responses Y_{in} and a second factor column of the corresponding factor levels.

```
> melon
                                             O before we apply lm, we need
  variety1 variety2 variety3 variety4
                                              to prepare our data in right form ->
     15.09
               17.12
                        21.20
                                  18.93
                                             we take each cal. & make It a 1-length
     20.21
              19.17
                        28.83
                                  31.34
                                              vect. -> we take 1st col. (sample), then put
     30.35 28.99
                        31.43
                                  30.13
                                              next sample after that ...
    25.03
                     25.90
            22.84
                                  23.18
5
     20.50
              31.72
                        21.98 19.86
     21.50
            18.67
                        24.48
                                  23.75
> melonframe=data.frame(yield=as.vector(as.matrix(melon)),
+ variety=factor(rep(1:4,each=6))) #create a data frame in the right format
> melonframe[1:5.]
  vield variety
                            > make sure that sample comes from right level
1 15.09
               1
2 20.21
3 30.35
               1
4 25.03
                       > declare that these are factors
5 20.50
> is.factor(melonframe$variety); is.numeric(melonframe$variety)
[1] TRUE
[1] FALSE
```

One-way ANOVA in R: testing

```
> melonaov=lm(yield~variety,data=melonframe)
> anova(melonaov)
```

Analysis of Variance Table

```
Response: yield

Df Sum Sq Mean Sq F value Pr(>F)

variety 3 43.55 14.516 0.5543 0.6512

Residuals 20 523.73 26.186

Var. doesn't make any diff. In yield
```

The command 1m creates an object of type linear model (many things can be extracted from it by using other functions), yield~variety is a model formula. Read it as: "explain yield using variety". The *p*-value for $H_A: \alpha_1=\alpha_2=\alpha_3=\alpha_4=0$ (which is the same as $H_A: \mu_1=\mu_2=\mu_3=\mu_4$) is 0.6512, hence H_A is not rejected, i.e., factor variety is not significant.

One-way ANOVA in R: estimation (1)

By default R uses treatment parametrization, i.e., $\alpha_1 = 0$. In this case, R reports the estimates of $\mu_1 = \mu + \alpha_1 = \mu$, $\alpha_2 = \mu_2 - \mu_1$, ..., $\alpha_I = \mu_I - \mu_1$.

```
> summary(melonaov)
```

[some output deleted]
Coefficients:

```
p-val.s for p=\mu_i p=\mu_i p=\mu_i p=\mu_i p=\mu_i
```

Estimate Std. Error t value Pr(>|t|)

```
22.1133 \mu = \mu_1 2.0891
                              10.585 1.21e-09 ***
(Intercept)
variety2
            0.9717
                       2.9545
                               0.329
                                       0.746
                                        0.247
variety3 ≪3 3.5233 2.9545
                               1.193
variety4 <
            2.4183
                       2.9545
                                        0.423
                               0.819
```

In the treatment contrasts, R takes the first level (here variety1, in alphabetical order) as a base level and compares the other levels to it. The estimates are $\hat{\mu}_1 = 22.1133$, $\hat{\alpha}_2 = 0.9717$, $\hat{\alpha}_3 = 3.5233$, $\hat{\alpha}_4 = 2.4.183$. The group means are found as $\hat{\mu}_i = \hat{\mu} + \hat{\alpha}_i$, $i = 1, \ldots, 4$ (remember $\hat{\alpha}_1 = 0$), they can also be obtained by command fitted(melonaov).

Command summary(model) also provides the output for testing individual $H_0: \mu_1 = 0$, $H_0: \alpha_i = \mu_i - \mu_1 = 0$, i = 2, 3, 4 (basically $H_0: \mu_i = \mu_1$ vs. $H_1: \mu_i \neq \mu_1$, i = 2, 3, 4). The test statistic $T_i = \frac{\hat{\alpha}_i}{s_{\hat{\alpha}_i}} \sim t_{n-l}$ under H_0 . The estimates $\hat{\alpha}_i$ (and $\hat{\mu}_1$) are given in column Estimate, the standard errors $s_{\hat{\alpha}_i}$ in Std. Error, the statistics values T_i in column t value, and the p-values in Pr(>|t|) (found as $P(|T| \geq |t|)$ for $T \sim t_{n-l}$).

One-way ANOVA in R: estimation (2)

```
| CI for μ<sub>2</sub>-μ<sub>1</sub> | CI for μ<sub>2</sub>-μ<sub>2</sub> | CI for μ<sub>2</sub>-μ<sub>1</sub> | CI for μ<sub>2</sub>-μ<sub>2</sub> | CI for μ<sub>2</sub>
```

```
Theory gives the following (1-\alpha)-Cl's: [\hat{\mu}\pm t_{n-l,\alpha/2}s_{\hat{\mu}}] and [\hat{\alpha}_i\pm t_{n-l,\alpha/2}s_{\hat{\alpha}_i}]. In this case, the 95% confidence intervals are for \mu_1: [17.755509, 26.471158]; for \mu_2-\mu_1: [-5.191228, 7.134561], for \mu_3-\mu_1: [-2.639561, 9.686228], for \mu_4-\mu_1: [-3.744561, 8.581228].
```

dep. en parameteriz. you use in lim

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One-way ANOVA in R: estimation (3)

An alternative to the (default) treatment parametrization is sum parametrization. This gives a decomposition of the population means into the overall mean μ and factor effects $\alpha_1, \alpha_2, \alpha_3, \alpha_4$ as

$$\mu_i = \mu + \alpha_i, \qquad i = 1, \dots, I,$$
 with the restiction $\sum_{i=1}^{I} \alpha_i = 0.$

 α_i 's are expressing the deviations from the mean, and their average is zero.

```
> contrasts(melonframe$variety) contr.sum  #to specify sum-parametrization
> melonaov=lm(yield~variety,data=melonframe); summary(melonaov)
[ some output deleted ]
                          > M here is diff b/c of paom -> but Mi
must be some
                                                                  do A before la
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 23. 8417
                          1.0446
                                  22.825 8.55e-16 ***

    you don't pet dy →

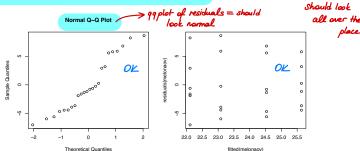
variety1 ≼ -1.7283
                         1.8092 -0.955
                                           0.351
                                                         to show that you work
variety242 -0.7567
                         1.8092 -0.418 0.680
variety3 4, 1.7950
                                                         w/ sum - param.
                         1.8092
                                0.992
                                            0.333
```

The 4 lines of the table give estimates of μ , α_1 , α_2 , α_3 , now in sum-parametrization. The estimate for α_4 is omitted, but could be computed from $\sum_{i=1}^4 \hat{\alpha}_i = 0$. We can compute the estimates for the μ_i 's: $\hat{\mu}_i = \hat{\mu} + \hat{\alpha}_i$, $i = 1, \ldots, 4$ (they must be the same as before).

One-way ANOVA in R: diagnostics

Use the data to check whether the assumption of normality is not totally untrue.

- The residuals $\hat{e}_{ik} = Y_{ik} \hat{\mu}_i$, $k = 1, ..., n_i$, i = 1, ..., I, are in a sense "estimated errors" e_{ik} (by using the data), hence should look normal.
- Another important plot for checking normality is the plot of the fitted values $\hat{Y}_{ik} = \hat{\mu}_i$ against the residuals \hat{e}_{ik} , it should show no pattern.
- > par(mfrow=c(1,2)); qqnorm(residuals(melonaov))
- > plot(fitted(melonaov),residuals(melonaov))



If the assumptions fail?

In this course, when applying any linear model (including all ANOVA models), you need to check normality of errors by using (at least) the following two tools: qqnorm plot of the residuals and the plot of fitted against residuals.

- The design of the experiment ensures that the data are independent random samples from the populations.
- However, the populations might be nonnormal or have different variances.
- If the number of data points is large, then the p-value should still be accurate.
- Otherwise, you may consider
 - transforming the data (e.g. use log Y);
 - using a different test;
 - omit some (outlying) data-points (careful!);
 - something else (there is no fix that always works).

Kruskal-Wallis test (a nonparametric counterpart of ANOVA test)

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Kruskal-Wallis test: design

The setting and design are the same as in the 1-way ANOVA (consider $n_i = N$, the balanced design). What if the normality assumption fails?

The Kruskal-Wallis test

- does not rely on the normality, it is based on ranks;
- is a nonparametric alternative to one-way ANOVA,
- is a generalization of the Mann-Whitney test for 2 samples;
- computes the sum of the ranks of Y_{i1},..., Y_{iN} for each i within the total data. Under H₀ these N ranks should all lie randomly between 1 and NI.

Data

sample 1:
$$Y_{11}, Y_{12}, ..., Y_{1N}$$

sample 2: $Y_{21}, Y_{22}, ..., Y_{2N}$
:
sample I : $Y_{I1}, Y_{I2}, ..., Y_{IN}$

Assume that these are sampled independently from I populations F_1, \ldots, F_I which are possibly different.

We test $H_0: F_1 = \ldots = F_I$ versus $H_1:$ at least two distributions are different.

> we test whether factors matter

Kruskal-Wallis test: setting and analysis

Setting: measurements Y_{ik} for i = 1, ..., I and $k = 1, ..., n_i$ from I different populations, Y_{ik} follows distribution F_i of population i.

Hypotheses: $H_0: F_1 = \ldots = F_k$ versus $H_1: F_i \neq F_j$ for some i, j.

Test statistic: $W = \frac{12}{n(n+1)} \sum_{i=1}^{I} n_i \bar{R}_i^2 - 3(n+1)$, where $N = n_1 + ... + n_I$ and

 $\overline{R}_i = \sum_{k=1}^{n_i} R_{ik}/n_i$ is the average pooled rank of the observations in sample i, R_{ik} is the rank (among all observations) of observation k from group i.

Distribution of W under H_0 : χ^2_{l-1} (approximately), the test is one sided.

-Assumption: all $n_i > 5$.

$$\rightarrow$$
 chi – sq. dist $w/dof = n-1$

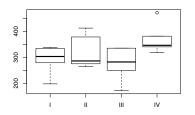
In R: kruskal.test(y,f,data=...), where y is the outcome, f is the factor.

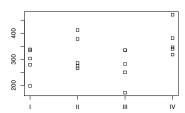
samples have to be at least 6 for valid.

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Analysis in R: data input and graphics

The dataset ratdata.txt contains the number of worms in rats in 4 different treatment groups.





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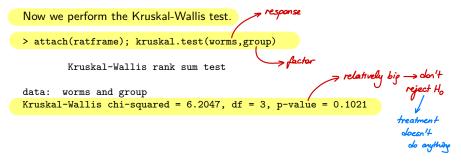
Analysis in R: data input

Create a data frame with the first columns containing all the outcomes $Y_{i,n}$ and the second column that indicates the levels of the factor factor.

```
> ratframe=data.frame(worms=as.vector(as.matrix(ratdata)),
                 group=as.factor(rep(1:4,each=5)))
> ratframe[1:6,]
  worms group
    279
    338
    334
             1
    198
             1
                                                                 ensure it's
a factor
5
    303
    378
> is.factor(ratframe$group); is.numeric(ratframe$group)
[1] TRUE
```

[1] FALSE

Analysis in R: testing (1)



The command kruskal.test performs the Kruskal-Wallis test and yields a p-value. The p-value for testing $H_0: F_1 = F_2 = F_3 = F_4$ is 0.1021, hence H_0 is not rejected.

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Analysis in R: testing (2)

Compare the result of Kruskal-Wallis test with the ANOVA test results:

```
> rataov=lm(worms~group); anova(rataov)
Analysis of Variance Table
```

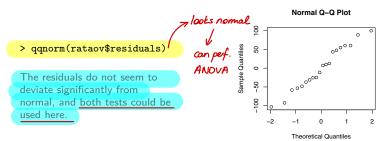
```
Response: worms

Df Sum Sq Mean Sq F value Pr(>F)

group 3 27234 9078.1 2.2712 0.1195

Residuals 16 63954 3997.1
```

The one-way ANOVA also does not yield a significant difference.



permutation tests in the setting of one-way ANOVA

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Setting and design

1-way ANOVA

⇒ Same as 1-way ANOVA Setting: an experiment with

- a numerical outcome Y.
- a factor that can be fixed at I levels ("label").

The same setting as 1-way ANOVA. The sample sizes for each label may differ.

EXAMPLE Medical experiment with outcome age at onset of a certain disease and label blood type.

EXAMPLE Quality of a genetic algorithm to determine the minimal value of a criterion function with outcome CPU time needed to find true minimum and label mutation probability set to 0.01, 0.02, 0.03, 0.04 or 0.05.

Design:

- Select I different labels
- Select n_i experimental units randomly from the population of label i.
- Perform the experiment $n_1 + n_2 + \ldots + n_l$ times, independently.

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Analysis

```
Data sample 1: Y_{11}, Y_{12}, ..., Y_{1n_1} sample 2: Y_{21}, Y_{22}, ..., Y_{2n_2} : sample I: Y_{I1}, Y_{I2}, ..., Y_{In_I}
```

Assume that these are sampled independently from I populations F_1, \ldots, F_I which are possibly different.

We test the null hypothesis $H_0: F_1 = F_2 = \cdots = F_I$ versus the alternative

 $H_1: F_i \neq F_j$ for some (i,j). \longrightarrow we test if all samples cone from some dist.

We choose a test statistic that expresses the conjectured differences between the I levels, and simulate the distribution of this statistic under H_0 .

The same null hypothesis as in the Kruskal Wallis test, the difference between the Kruskal Wallis test and permutation tests is in the test statistic.

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Analysis in R: data input and graphics

The dataset dogs.txt concerns measures of plasma epinephrine in dogs for three different anesthesia drugs ("iso","halo","cyclo").

```
> dogs=read.table("dogs.txt",header=TRUE)
```

- > treat=factor(rep(1:3,c(10,10,10)),labels=c("iso","halo","cyclo"))
- > dogsdata=data.frame(plasma=as.vector(as.matrix(dogs)),treat)

```
> head(dogsdata)
  plasma treat
1   0.28   iso
2   0.51   iso
3   1.00   iso
4   0.39   iso
5   0.29   iso
6   0.36   iso
> boxplot(plasma~treat,data=dogsdata)
```

this treatment looks cliff then others

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Analysis in R: testing (1)

1-way ANOVA

```
, choice of stat.
> attach(dogsdata)
> mystat=function(x) sum(residuals(x)^2)
> B=1000
                                       reshuffle col. of labels -> if all dist are some,

A shouldn't matter
> tstar=numeric(B)
> for (i in 1:B) {
    treatstar=sample(treat)
                                   ## permuting the labels
    tstar[i]=mystat(lm(plasma~treatstar)) }
> myt=mystat(lm(plasma~treat))
                                      > Compute residuals of 1-way ANOVA model for
                                            for each reshuffled ver. of labels
```

The above test statistic is the sum the squared residuals which measures the fit of one-way ANOVA model to the observed data:

 $\sum_{i=1}^n \sum_{k=1}^{n_i} \hat{e}_{ik}^2 = \sum_{i=1}^n \sum_{k=1}^{n_i} (Y_{ik} - \hat{\mu}_i)^2$, where $\hat{\mu}_i$ is the average per label. In R, this can be programmed efficiently as sum(residuals($lm(data \sim labels))^2$). Note that we do not use the p-values of lm, we find p-values in a bootstrap fashion.

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Frequency

Analysis in R: testing (2)

> hist(tstar) > myt [1] 2.72474 > pl=sum(tstar<myt)/B</pre> > pr=sum(tstar>myt)/B > 2*min(pl,pr) [1] 0.022 -> small = reject The treatment is clearly significant. This is (hopefully) in line with your results using 1-way ANOVA and Kruskal-Wallis test in the corresponding assignment.

Histogram of tstar 8 20 2.5 3.0 3.5 tstar

Discussion

- A permutation test for independent samples can be performed with any test statistic that expresses difference between the samples.
- An alternative to the permutation test for independent samples is the Kruskal-Wallis test.
- Nearly all hypotheses concerning the dependence of some quantity on different levels of a "treatment" can be investigated using some sort of permutation.
- By permuting the categories of either the row or column factor in a contingency table (considered later on), one can test the null hypothesis of no dependence between these two factors.
- In fact a permutation test is a bootstrap test, because the distribution of the test statistic is approximated by simulation.

To finish

Today we learned:

- one-way ANOVA
- Kruskal-Wallis test
- permutation tests in the setting of one-way ANOVA

Next time: 2-way ANOVA, factorial design, multiple comparisons.

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