Using Statistics in Ecology 2019, homework part two

"Generate a data set" means that you should also describe briefly a biologically realistic setting which may have yielded such data, i.e. the variables must have names and the results should be meaningfully interpreted. Everything which is in the figures must be readable. Everyone will do his/her work independently, you are allowed to ask for advice but direct similarities will be punished. Excessive numerical accuracy is moderately punishable. Please copy the text of each task before you answers. Display your "a" value.

 $A \leftarrow 8$, my code is available on github: https://github.com/lycanea/Using Stat in Eco HW script and data

1. Generate biologically relevant data in which chi-squared test would reveal a statistically significant association in a 2x2 (two times two) frequency table. In one of the cells there should be value 6a. Present **the table and the result b**oth as copy-paste from R and as such a **sentence** which you would be OK for a research paper. Present a **table** with identical marginal distributions in which there is no significant association between the variables (this can you do by hand at home).

Data description: I would like to compare frequency of flower phenotypes, which have 2 colour forms (red vs. blue) and forms with different number of anthers (five vs. three) in population.

	Red	Blue	Row marginals	
Five Three	48 10	18 40	66 50	
Collum marginals	58	58	N =116	

A Chi- square test of independence was calculated comparing the frequency of flower phenotypes in population. A significant interaction was found (χ 2 (1) = 29.6, p < .001). Red flowers were more likely to have five anthers (41%) than blue flowers (16%) and blue flowers were more likely to have three anthers (34%) than red flowers (9%).

```
(petal_colour.anther_number.nonsignificat <- matrix(c(a*6, 34,42,40),2,2,dimnames =
list(c("five", "three"),c("red", "blue"))))
    red blue
five  48   42
three  34   40</pre>
```

chisq.test(petal_colour.anther_number.nonsignificat)

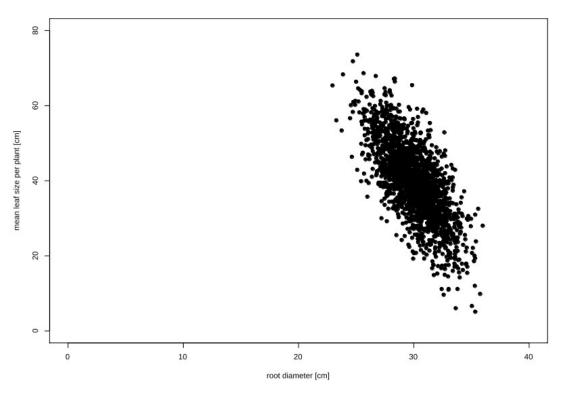
Pearson's Chi-squared test with Yates' continuity correction

data: petal_colour.anther_number.nonsignificat X-squared = 0.61562, df = 1, p-value = 0.4327

	Red	Blue	Row marginals	
Five Three	48 34	42 40	90 74	
Collum marginals	82	82	N =164	

A Chi- square test of independence was calculated comparing the frequency of flower phenotypes in population. A interaction was not significant (χ 2 (1) = .62, p = .4).

2. Generate a data set in which two continuous independent variables are negatively correlated (-0.65>r>-0.85, **present a graph, and a result of correlation analysis)** and the dependent variable (sample mean between 5a and 6a) depends on both of these variables, when examined one at a time (present graphs). Perform type I analyses with both orders of the variables (do not include the interaction), present results as raw tables (copy-paste from R). Explain the difference in the interpretation of the results. Present the results also for type III analysis but this time as an edited ANOVA table (as you would present it in a research paper). Please do not draw more lines than it is customary for tables in research papers.



Graph of relationship between two independent variables mean leaf size per plant and root diameter. There is a significant strong negative correlation ($r_{(1998)}$ = -.7, p = < .001, Pearson).

```
data: df$x and df$y
   t = -43.814, df = 1998, p-value < 2.2e-16
   alternative hypothesis: true correlation is not equal to 0
   95 percent confidence interval:
    -0.7216883 -0.6769387
   sample estimates:
    cor
   -0.7
   There is a significant strong negative correlation between two independent
   variables mean leaf size per plant and root diameter (r_{(1998)} = -.7, p = < .001,
   Pearson).
anova(lm(herbivory~root_dia+m_leaf_size, data=df))
Analysis of Variance Table
Response: herbivory
          Df Sum Sq Mean Sq F value Pr(>F)
          1 233739 233739 7718.1305 <2e-16 ***
root dia
                    35 1.1704 0.2794
m leaf size 1
                35
Residuals 1997 60478
                        30
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> # Type I linear model - changed order
> anova(lm(herbivory~m_leaf_size+root_dia, data=df))
Analysis of Variance Table
Response: herbivory
          Df Sum Sq Mean Sq F value Pr(>F)
m_leaf_size 1 111673 111673 3687.5 < 2.2e-16 ***</pre>
root dia 1 122102 122102 4031.8 < 2.2e-16 ***
Residuals 1997 60478
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Pearson's product-moment correlation

There is difference in ANOVA Type I with different order. It is because predictors (independent variables) are correlated. It means, that we can not answer the question which variable has a direct effect and which has an indirect (mediated) effect. In this example we cannot claim a direct (not mediated by root diameter) effect of mean leaf size on herbivory of plant.

ANOVA type III table for herbivory rate

Source	df	Type III SS	F	р	
Mean leaf size	1	35	1.2	non-significant	
Root diameter	1	122102	4031.9	<.001	

And for variables in opposite order:

ANOVA type III table for herbivory rate

Source	df	Type III SS	F	p
Root diameter	1	122102	4031.9	<.001
Mean leaf size	1	35	1.2	non-significant

- 3. Generate data in which the results of a type I ANOVA with two discrete independent variables do not depend at all on the order of the variables in the model (recall **which assumption should be met** to achieve this). Do <u>not</u> include an interaction in the model. Please present the results with the two different orders as raw tables. Calculate the residuals and present graphically frequency distributions of the **residuals for both levels** of one of the independent variables. Are the assumptions of ANOVA met? (Tell why. No worries if not).
- 1. Which assumption should be met there should not be association between independent variables in case of two discrete variables, they should have equal frequency in (see table).

```
high low
blue 200 200
red 200 200
```

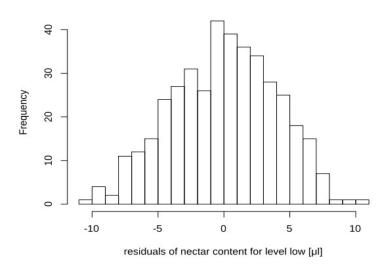
Generate data:

I generate data with scenarios when I want to compare rate of flower visitation (measured as number of flower visitors per hour) on flowers which differs in a) petal colour (red, blue) and nectar content (low, high).

Output of I ANOVA:

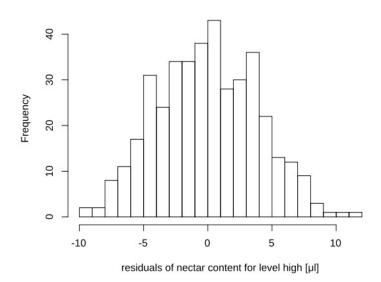
```
> mod1 <- (lm(f visi~nectar content+petal colour, data = fl visitors))</pre>
> anova(mod1)
Analysis of Variance Table
Response: f_visi
               Df Sum Sq Mean Sq F value
                                             Pr(>F)
nectar content 1 5269.7 5269.7 341.70 < 2.2e-16 ***
                1 5630.2 5630.2 365.07 < 2.2e-16 ***
petal colour
Residuals
              797 12291.4
                             15.4
> mod2 <- (lm(f_visi~petal_colour+nectar_content, data = fl_visitors))</pre>
> anova(mod2)
Analysis of Variance Table
Response: f visi
               Df Sum Sq Mean Sq F value
                                             Pr(>F)
               1 5630.2 5630.2 365.07 < 2.2e-16 ***
petal colour
nectar_content 1 5269.7 5269.7 341.70 < 2.2e-16 ***
              797 12291.4
Residuals
                             15.4
```

histogram of residuals



Histogram of residuals of nectar content for level low $[\mu l]$ from the second ANOVA model.

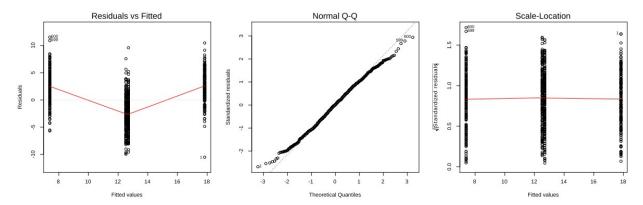
histogram of residuals



Histogram of residuals of nectar content for level high $[\mu l]$ from the second ANOVA model.

Assumptions of ANOVA are met:

- 1. Independence of observations I measured randomly selected flowers across the galaxy (trust me).
- 2. Normality in distribution of residuals residuals are in this case normally distributed as you can see on the second diagnostic plot or on histogram of residuals.
- 3. Homoscedasticity variance in levels of predictors should be more or less equal.



Diagnostic plots of the second ANOVA model. It is clear that assumptions of ANOVA are met.

4. Examine section "Analysis of covariance — one important application" (topic 7) and present an example from the ecology of fish (what was studied and why?) in which the described logic applies. Discuss the setting and explain, how would you interpret the results of the analyses in which the covariate 1) is not, 2) is included in the model and in the last case 1) will or 2) will not attain significance. No need to perform the analysis but present a hypothetical table of results.

Our study investigate influence of nitrogen concentration in water on reproduction of fish. Nitrogen concentration in water was for long time in suspicion for negative impact on fish populations. In this study was selected one species and number of eggs laid per female was measured by using a diving cylinder in artificial ponds, which do not differ in anything else than in nitrogen content. Length of females was used as covariate (all females in analysis was measured underwater in order to avoid any harm). Fortunately is length of females normally distributed in all ponds.

Results of ANOVA with covariate is not included:

Table
ANOVA Summary Table for Variable 1

Source	df	MS	F	p
Nitrogen conc. Total	1 99	220.22	2.56	.06

 $R^2 = .18$

There is not a significant relationship between fish reproduction (number of eggs per female) and nitrogen concentration $(F(1,99)=2.56,\ p=.06)$.

#Results of ANOVA with included covariate: Table

ANOVA Summary Table for Variable 1

Source	df	MS	F	p	
Female length Nitrogen conc. Total	1 1 98	420.02 30.22	7.03 3.56	.01 .02	

 $R^2 = .29$

There is a significant effect of nitrogen concentration in water on fish reproduction after controlling for the effect of fish length (F(1,98) = 3.56, p = 0.2)

#Results of ANOVA with included covariate:

Table

ANOVA Summary Table for Variable 1

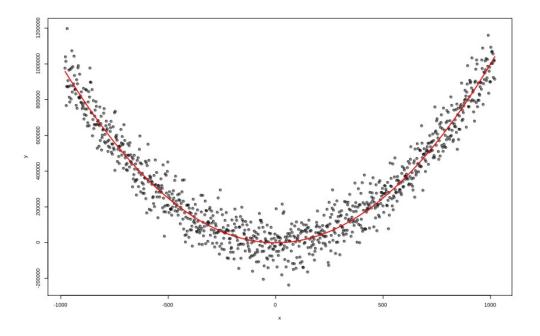
Source	df	MS	F	р	
Female length Nitrogen conc. Total	1 1 98	120.02 230.22	1.03 1.56	.1 .2	

 $R^2 = .29$

There is not a significant effect of nitrogen concentration in water on fish reproduction after controlling for the effect of fish length (F (1,98) = 1.56, p = 0.2)

5. Generate data in which the relationship between two variables deviates significantly from linearity and the relationship is convex. The mean of the sample of the independent variable should be between 2a and 3a. Present the test of non-linearity (raw table), the graph and the equation of the relationship (edited).

Analysis of Variance Table



```
Coefficients:
(Intercept) x I(x^2)
-528.2689326 1.3977949 0.9991705
```

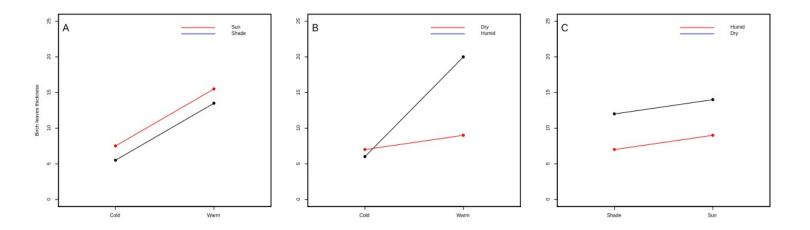
Equation:

```
y = -528.2689 + 1.3977949 x - 0.9991705x^2
```

6. The thickness of birch leaves in shade, humid and warm conditions is a. Please propose the values of leaf thickness for all combinations of illumination (shade/sun), humidity (humid/dry) and temperature (warm/cold) so that there were one and only one two-factor interaction in the data, a three-way interaction should not be there either. Present the situation graphically (you may draw by hand). How would you verbally formulate a three-way interaction if it still was there?

Mean values of birch leaves thickness in different conditions

	Sh	Shade		Sun		
	humid	dry	humid	dry		
Warm Cold	8	19	10	21		
Cold	6	5	8	7		



Graphs describing relations between variables. Only graph B shows two-way interaction between humidity and temperature.

How to formulate three-way interaction:

Thickness is dependent on levels of all three predictors. Ergo: thickness in different shade condition dependents on levels of humidity and temperature, in different humidity levels dependents on levels of temperature and shade and in different temperature levels dependents on levels of humidity and shade.

7. Describe a fictional situation from bird ecology in which you would use a mixed ANOVA with one random (not "brood", invent something different) and one fixed factor. Explain, why are the factors treated as fixed/random. Generate the data and present the result as a raw table. Would it be reasonable to alternatively treat one of the random factors as fixed. If not, then why? If yes, how would it change the interpretation of the results?

Birds of two closely related sympatric species were observed in Spain and amount of mites per bird was counted. Aim of the study was investigate if there is difference in bird species infestation. For the analyses was used species as fixed effect and study site identity was used as random effect.

Species identity was treated as fixed factor, because aim of the analyses was investigated difference between species.

Study site identity was treated as random effect, because birds from different sites differs in the number of mites (probably because some sites were more suitable for mites of species A and some for mites of species B).

Analysis with fixed and random factors

Analysis with both factors as fixed

```
Response: mites

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

spec 1 47415 47415 625.060 < 2.2e-16 ***

site 3 9439 3146 41.477 < 2.2e-16 ***

Residuals 395 29963 76
```

It could be reasonable to treat one of the random factor as fixed – in scenario where we want to test difference between sites. If there is difference between sites

8. There were 6 turtles, 3 were fed with snails, 3 were not (two treatments). Shell thickness on the turtles was measured in one, two, three and four years starting from the beginning of the treatment. The researchers are interested whether there is some overall effect of the treatment on shell thickness, and whether the dynamics of shell thickness in time differs between the treatments. Please find the data as an excel sheet, first please analyse the data with an ordinary ANOVA (treatment and time) not accounting for the fact that the same individuals were repeatedly measured, and then as a correct repeated measurements ANOVA. Present the results of **both analyses as raw tables,** find the differences (also in degrees of freedom) and explain what do they come from. Please note that in repeated measurements ANOVA, the number of error degrees of freedom may differ for different effects. Formulate the result of the repeated measurements ANOVA as a biologically meaningful sentence.

Analysis without repeated measuremens

anova(turmod)

Analysis of Variance Table

Response: shell

```
Df Sum Sq Mean Sq F value Pr(>F)
treatment 1 2.042 2.0417 1.0515 0.3174
time 1 1.008 1.0083 0.5193 0.4795
treatment:time 1 0.075 0.0750 0.0386 0.8462
Residuals 20 38.833 1.9417
```

Analysis with repeated measuremens

> anova(turmod2)

```
numDF denDF
                            F-value p-value
                       16 142.79091 <.0001
(Intercept)
                  1
treatment
                  1
                        4
                            2.96350 0.1603
time
                  1
                       16
                            0.54053 0.4729
                  1
                            0.01608 0.9007
                       16
treatment:time
```

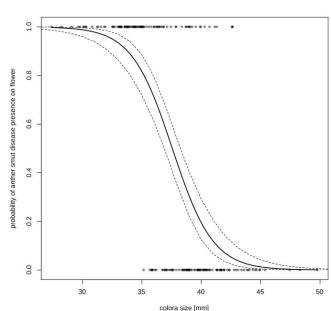
Effect of turtle diet does not change in time (p=.9). Furthermore, there is no difference between turtle diet (p=.16) and shell thickness does not change in time (p=.47).

The differences comes from the fact, that measurements of individuals are not treated as independent observations, because they would be affected from previous state.

9. Generate data which you would analyse using the method of logistic regression and where the parameter b is in the range -0.7<b<-0.5. Perform the analysis, present the figure, significance test in the raw form and the equation of the logistic function (edited). Present the result as a biologically informative sentence as you would do it in a research paper.

How is presence of anther smut disease in flowers of Dianthus carthusianorum affected by corolla size?

```
glm(formula = y \sim x1, family = binomial)
Deviance Residuals:
     Min
                 1Q
                       Median
                                       3Q
                                                Max
-1.80250 -0.67773
                      0.01658
                                 0.55096
                                            2.45444
Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) 21.90020
                         2.97940
                                    7.351 1.97e-13 ***
             -0.58309
                          0.07909 -7.373 1.67e-13 ***
x1
                                                              1 flower
(Dispersion parameter for binomial family taken to be 1)
                                                               smut disease presence on
    Null deviance: 277.26 on 199 degrees of freedom
Residual deviance: 166.18 on 198 degrees of freedom
                                                                 9.0
AIC: 170.18
Number of Fisher Scoring iterations: 5
Analysis of Deviance TableModel: binomial, link: logit
                                                                 0.4
                                                               probability of anther
Response: y
Terms added sequentially (first to last)
                                                                 0.2
     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL
                          19
                                 27.726
                          18
                                 15.864 0.000573 ***
x1
      1
         11.862
                                                                 0.0
Call: glm(formula = y \sim x1, family = binomial)
Coefficients:
(Intercept)
                       x1
    -12.039
                    1.082
Degrees of Freedom: 19 Total (i.e. Null); 18 Residual
Null Deviance:
                     27.73
Residual Deviance: 15.86
                                  AIC: 19.86
```



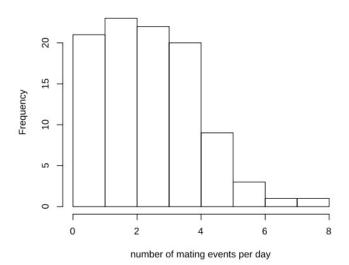
Flower corolla size had significant effect on presence of anther smut disease in flowers of Dianthus carthusianorum (p < .001)

10. Present a fictional case in which you would compare a Poisson distributed variable between two populations of monkeys (in one of the groups, let it be $\mu = 1 + a/10 \ (\pm 10\%)$) Generate respective data and present these distributions graphically. Present tests to show that the generated distributions do not deviate significantly from Poisson distribution. Present a test for the among-population differences in the values of these variables. Present the results of the test both as raw output and as a meaningful sentence.

```
gf = goodfit(ska[ska$group == "B",]$mat freq,type= "poisson",method= "ML")
> gf.summary = capture.output(summary(gf))[[5]]
> pvalue = unlist(strsplit(gf.summary, split = " "))
> pvalue = as.numeric(pvalue[length(pvalue)]); pvalue
[1] 0.09868836 - I do not rejected H0
gf = goodfit(ska[ska$group == "A",]$mat_freq,type= "poisson",method= "ML")
> gf.summary = capture.output(summary(gf))[[5]]
> pvalue = unlist(strsplit(gf.summary, split = " "))
> pvalue = as.numeric(pvalue[length(pvalue)]); pvalue
[1] 0.9112508 - I do not rejected H0
Model:
mat freq ~ group
       Df Deviance
                      AIC
                             LRT Pr(>Chi)
<none>
           224.45 705.78
group
            253.84 733.16 29.384 5.938e-08 ***
```

Histogram of mating frequency in group A

Histogram of mating frequency in group B



There is a significant difference between apes groups in mating frequency per day (p < .001).11. Describe a situation when, studying domestic cats, you should "merge" three variables into one principal component (please avoid overlap with the example presented in the lecture, invent something by yourself). Present the interpretation of the new variable. Why cannot it be measured directly? Generate the data and find out whether the new variable correlates with some fourth one (present raw results of the correlation analysis). Present a table showing the values of the original variables and the calculated PCA component score.

I studied relation between number of positive values obtained by cat's pictures on internet and their cuteness which was described by using principal component from their hair softness score, number of stroking events per day and number of events when they are not trying kill their owner.

> cor.test(cuteness\$x[,1], df\$n_likes)

- it is possible to use Pearson correlation test, because both variables are not significantly different from normal distribution.

```
Pearson's product-moment correlation

data: cuteness$x[, 1] and df$n_likes
t = 12.905, df = 18, p-value = 1.552e-10
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
0.8755055 0.9803668
sample estimates:
cor
0.9499772
```

Highly significant and strong correlation between numbers of positive marks on internet and PC1 which describes cuteness of cats (number of likes) (r(df = 18) = .9, p < .001)

30 40 40 18 -3.87753444262232 29 50 32 8 -9.41787610145726	2.26612088256017 0.185533802586723 16.2380916845021 -5.98606616930556 -3.54611270350631 -10.0778976822881 -0.494326619589397 0.387402263259279
29 50 32 8 -9.41787610145726	-3.54611270350631 -10.0778976822881
33 50 67 23 22.8597039132042	-0.494326619589397 0.387402263259279
29 36 39 17 -7.09299377411167	
30 26 43 20 -7.8154736058258	-11.4549444733282 2.47135983410118
32 54 63 21 21.0466822444033	2.16232014659075 -10.4339128847602
31 55 40 23 6.11345304287751	14.3319670821343 2.32925126363622
26 17 20 2 -37.5254360959346	-4.96338098298931 -0.558117395922184
33 47 60 30 19.7625998900167	-3.56387831979698 -0.208367245006227
34 50 63 38 27.3101013763447	-3.65763426325821 4.79427517045207
31 42 49 33 10.8560792038285	-2.69954956582022 8.4476610053838
30 42 35 30 -0.594059308351117	4.78828566557109 12.6698229633664
28 34 33 19 -11.4076584523846	0.544930232708102 5.31727182634269
31 28 48 18 -4.21432642887387	-11.9897735607986 -1.9722532067842
30 41 52 23 7.6232423957735	-3.64505047465649 -1.48485097315791
28 37 34 9 -14.0525529308084	4.01510896177335 -4.22014883054072
28 28 42 15 -9.95632493048511	-8.53736044802398 -1.64014342638571
28 36 36 5 -15.0771202528146	2.72234277014007 -8.49554558130516
30 41 44 22 1.42813788731013	0.532320485230913 1.54339142016304
30 47 40 27 4.03135636991082	6.95052350055687 6.93133384616455

Raw table with original data and PCA output.



Map of the Kihnu island. Points represent sites, numbers represent mean number of seeds and size of points represent number of seeds on the site.



Map of the Kihnu island. Points represent sites, numbers represent mean number of seeds and size of points represent number nutritient content on sites.

12. Describe a fictional ecological study in which you have studied the dependence of one continuous variable on another, so that the values of both variables have recorded from 20 sites on Kihnu island. There should be spatial autocorrelation (SAC) in the independent variable but not in the dependent variable. Present a map displaying the values of both variables for all study sites. Present a verbal interpretation of the SAC. What may have caused the SAC?

We studied relation between plant seed production and nutrient content on 20 sites on Kihnu island. The nutrient content is strongly spatially correlated, possibly because there is some historical influence in management of sites and the northern part of the Kihnu island is more nutrient rich. Seed production is independent on nutrient content, because it is strongly affected by other factors (number of mates, herbivory rate etc.).