Multivariate Statistics in Ecology and Quantitative Genetics
Some remarks on balanced design and on parameter transformations.

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http://evol.bio.lmu.de/_statgen

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Contents

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The aim of the study was to assess how the sportiveness depended on gender and smoking behavior. Thus, the students were subdivided into four groups:

	male	female	$\sum$
smoker	18	9	27
non-smoker	30	43	73
$\sum$	48	52	100

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	male	female	$\sum$
smoker	18	9	27
non-smoker	30	43	73
$\sum$	48	52	100

(Smoking seems to be gender-specific, $p = 0.026$, Fisher's exact test)

```
> t.test(runtime[smoking=="s"],runtime[smoking=="n"])

Welch Two Sample t-test

data:  runtime[smoking == "s"] and runtime[smoking == "n"
t = 0.1102, df = 60.611, p-value = 0.9126
alternative hypothesis: true difference in means is not e
95 percent confidence interval:
 -7.522165  8.399714
sample estimates:
mean of x mean of y
 91.06888  90.63010
```

```
> drop1(lm(runtime~smoking+sex),test="F")
Single term deletions

Model:
runtime ~ smoking + sex
        Df Sum of Sq   RSS    AIC F value     Pr(F)
<none>                20570 538.64
smoking  1    1078.7 21648 541.75   5.087   0.02635 *
sex      1   18548.6 39118 600.92  87.469 3.356e-15 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

In another (hypothetical) survey, a balanced design was used, that is, equal numbers of students were selected for the four groups:

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	male	female	$\sum$
smoker	25	25	50
non-smoker	25	25	50
$\sum$	50	50	100

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	male	female	$\sum$
smoker	25	25	50
non-smoker	25	25	50
$\sum$	50	50	100

Balanced design, but no representative sampling!

```
> drop1(lm(runtime~smoking+sex),test="F")
Single term deletions

Model:
runtime ~ smoking + sex
        Df Sum of Sq   RSS    AIC F value     Pr(F)
<none>                23691 552.77
smoking  1    3084.3 26776 563.01  12.628 0.0005889 ***
sex      1   10648.1 34339 587.89  43.597 2.158e-09 ***
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

```
> t.test(runtime[smoking=="s"],runtime[smoking=="n"])

Welch Two Sample t-test

data:  runtime[smoking == "s"] and runtime[smoking == "n"
t = 2.9669, df = 94.736, p-value = 0.003808
alternative hypothesis: true difference in means is not e
95 percent confidence interval:
  3.674649 18.539956
sample estimates:
mean of x mean of y
 101.1723   90.0650
```

Note that the linear model commands

```
summary(lm(runtime~smoking+sex))
```

and

```
drop1(lm(runtime~smoking+sex),test="F")
```

are neither restricted to represenative sampling nor to balanced design.

But how to interprete the group means?

Representative sampling:

```
> mean(runtime[sex=="male"])
[1] 76.99001
> mean(runtime[sex=="female"])
[1] 103.4488
> mean(runtime[smoking=="s"])
[1] 91.06888
> mean(runtime[smoking=="n"])
[1] 90.6301
```

Balanced design:

```
> mean(runtime[sex=="male"])
[1] 85.29967
> mean(runtime[sex=="female"])
[1] 105.9376
> mean(runtime[smoking=="s"])
[1] 101.1723
> mean(runtime[smoking=="n"])
[1] 90.065
```

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[1] 90.065
```

In the balanced design, smokers are overrepresented (compared to reality), and females are overrepresented among the smokers and underrepresented among the non-smokers.

Let i be the index for the row of a data table. The data are subdivided into groups and G_i is the group row i (or patient i) belongs to; e.g. G_i can be the treatment of patient i. Let Y_i be the response variable, e.g. the blood pressure of patient i. We can apply an anova to check whether Y depends on G, and the model behind it is:

$$Y_i = b_{G_i} + \varepsilon_i$$

where the ε_i are assumed to be independent and normally distributed with expectation 0, and all ε_i have the same variance σ^2. During the ANOVA we estimate the influence b_{G_i} of the group on Y_i by the group mean $\widehat{b_g}$. Thus, the residuals $r_i := Y_i - \widehat{b_{G_i}} \approx Y_i - b_{G_i} = \varepsilon_i$ should be approximately normally distributed.

More than one factor can play a role. For example we may take into account that the blood pressure Y_i of a patient may depend on the sex S_i of the patient. In this case the model behind the anova takes the form

$$Y_i = b_{G_i} + c_{S_i} + \varepsilon_i.$$

b_{G_i} depends only on the treatment group and c_{S_i} only on the sex of the female. If we also want allow in *interaction* between the treatment and the sex, we need another variable d_{G_i,S_i} that may depend on both:

$$Y_i = b_{G_i} + c_{S_i} + d_{G_i,S_i} + \varepsilon_i.$$

This makes possible, for example, that a certain treatment has a stronger effect for males than for females.

A *balanced design* means, that the sample size are the same for each combination of factors. E.g. 10 males and 10 females in each treatment group. Some ANOVA-based method will only work for balanced designs. Therefore, it is preferable to use a balanced design when planning an experiment. If the data, however, are observations from nature, the “design” is usually unbalanced and this has to be taken into account in the analysis.

One of the methods for which you need a balanced design is Tukey’s HSD (honest significat differences). From an anova it computes confidence intervals for the pairwise differences between the group means with mulptiple-testing correction (see slides on ANOVA in the EES&MEME basic statistics course).

Contents

Tne npk dataset from the MASS[1] library: Yield of peas that grew with or without application of nitrogen (N), phosphate (P), and potassium (K).

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We compensate for effects of the block and randomize within and between the blocks.

Balanced design: Each treatment appears three times.

---

[1] Venables, W.N. and Ripley, B.D. (2002) Modern Applied Statistics with S. Fourth edition. Springer

Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
PK 49.5	N 59.8	P 62.8	N 62	NP 52	NK 57.2
NP 62.8	NPK 58.5	NPK 55.8	NPK 48.8	— 51.5	NP 59
— 46.8	K 55.5	N 69.5	K 45.5	NK 49.8	PK 53.2
NK 57	P 56	K 55	P 44.2	PK 48.8	— 56

- Note the balance within the blocks: Any substance apears twice in each block.
- Cannot estimate triple interaction N:P:K because it is confounded with block differences.

```
> (npk.aov <- aov(yield~block + N*P*K,data=npk))
Call:
   aov(formula = yield ~ block + N * P * K, data = npk)

Terms:
                   block        N        P        K      N:P      N:K      P:K
Sum of Squares  343.2950 189.2817   8.4017  95.2017  21.2817  33.1350   0.4817
Deg. of Freedom        5        1        1        1        1        1        1
                Residuals
Sum of Squares   185.2867
Deg. of Freedom        12

Residual standard error: 3.929447
1 out of 13 effects not estimable
Estimated effects may be unbalanced
```

```
> summary(npk.aov)
            Df Sum Sq Mean Sq F value   Pr(>F)
block        5 343.29  68.659  4.4467 0.015939 *
N            1 189.28 189.282 12.2587 0.004372 **
P            1   8.40   8.402  0.5441 0.474904
K            1  95.20  95.202  6.1657 0.028795 *
N:P          1  21.28  21.282  1.3783 0.263165
N:K          1  33.13  33.135  2.1460 0.168648
P:K          1   0.48   0.482  0.0312 0.862752
Residuals   12 185.29  15.441
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

Giving *p* values for variables that are also involved in interaction terms makes sense only if the design is balanced. It refers to a coefficient that is averaged over the different cases of interaction.

From the R manual page of "aov":

" *'aov' is designed for balanced designs, and the results can be hard to interpret without balance: beware that missing values in the response(s) will likely lose the balance.*"

The command `drop1(lm(...),test='F')` does not assume a balanced design and therefore does not report *p* values for variables that are involved in interactions.

```
> drop1(lm(yield~block +(N+P+K)*(N+P+K),data=npk),test="F")
Single term deletions

Model:
yield ~ block + (N + P + K) * (N + P + K)
       Df Sum of Sq    RSS    AIC F value   Pr(F)
<none>              185.29 73.052
block   5    343.30 528.58 88.211  4.4467 0.01594 *
N:P     1     21.28 206.57 73.662  1.3783 0.26317
N:K     1     33.14 218.42 75.001  2.1460 0.16865
P:K     1      0.48 185.77 71.115  0.0312 0.86275
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

Contents

Be careful with the interpretation of ANOVA tables! The R command anova, applied to a single model gives a so-called “Type I Anova”, where each line take only the variables in the lines above into account. Example: Chill coma recovery times measured by different persons on different days for different fly lines.

```
> anova(model4)
Analysis of Variance Table

Response: log(ccrt)
           Df  Sum Sq Mean Sq F value    Pr(>F)
line        1  1.2224 1.22238 13.1486 0.0003812 ***
day        11  2.8471 0.25883  2.7841 0.0023769 **
person      1  0.0850 0.08504  0.9147 0.3402393
[...]
```

For example, the p-value 0.0023769 tells how much better the model with line and day can explain the data compared to a model that only takes line into account. Thus, the values assigned to variables depend on the input order.

If you use the R command drop1 with the option test="F", you get a so-called "Type II Anova", in which each line shows the influence of one variable, given the estimates of *all* other variables.

```
> drop1(model4,test="F")
[...]
      Df Sum of Sq    RSS     AIC F value    Pr(F)
<none>              15.618 -418.91
line   1   0.05860 15.677 -420.23  0.6304 0.428338
day   11   2.47080 18.089 -414.18  2.4161 0.008177 **
person 1   0.08504 15.703 -419.92  0.9147 0.340239
```

For example, the *p*-value 0.008177 says that a model that takes line, day and person into account explains the data significantly better than a model that uses only line and person.

Back to the footrace example with non-balanced design:

```
> summary(aov(runtime~sex+smoking))
            Df  Sum Sq Mean Sq F value    Pr(>F)
sex          1 17473.7 17473.7  82.400 1.316e-14 ***
smoking      1  1078.7  1078.7   5.087   0.02635 *
Residuals   97 20569.7   212.1
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
> summary(aov(runtime~smoking+sex))
            Df  Sum Sq Mean Sq F value    Pr(>F)
smoking      1     3.8     3.8  0.0179    0.8939
sex          1 18548.6 18548.6 87.4693 3.356e-15 ***
Residuals   97 20569.7   212.1
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

But for the dataset with *balanced design* (for which aov is more appropriate) the input order does not matter even for Type I anova:

```
> summary(aov(runtime~sex+smoking))
            Df  Sum Sq Mean Sq F value    Pr(>F)
sex          1 10648.1 10648.1  43.597 2.158e-09 ***
smoking      1  3084.3  3084.3  12.628 0.0005889 ***
Residuals   97 23691.2   244.2
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
> summary(aov(runtime~smoking+sex))
            Df  Sum Sq Mean Sq F value    Pr(>F)
smoking      1  3084.3  3084.3  12.628 0.0005889 ***
sex          1 10648.1 10648.1  43.597 2.158e-09 ***
Residuals   97 23691.2   244.2
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

Contents

It is often important to rescale (i.e. transform) the data. For example, if a comparison between fitted values (group means) and the residuals show that the larger values have larger standard deviations, this may mean that the random error ist rather multiplicative than additive (as it should be). In this case, a log transform may help. Sometimes, there is a good explantation why a certain transformation should be applied. Sometimes the Box-Cox-Transform can help, which can take various shapes, depending on a parameter to be optimized. Other transformations are also possible, not only for the target variable but also for explanatory variables in regression models.

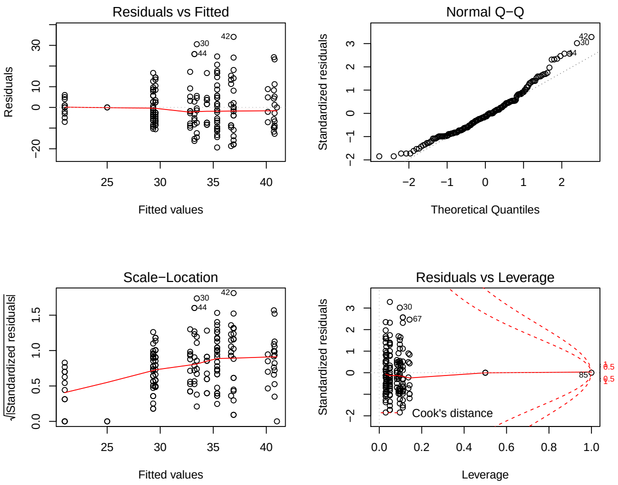
Back to the example with chill coma recovery times with simulated data motivated by

N. Svetec, A. Werzner, R. Wilches, P. Pavlidis, J.M. Alvarez-Castro, K.W. Broman, D. Metzler, W. Stephan (2011) Identification of X-linked quantitative trait loci affecting cold tolerance in Drosophila melanogaster and fine mapping by selective sweep analysis.
Molecular Ecology **20**(3):530-544

```
> fly <- read.table("CCRT.txt",h=T)
> str(fly)
'data.frame': 182 obs. of  7 variables:
 $ line   : Factor w/ 2 levels "A","B": 1 1 1 1 1 1 1 1 1
 $ day    : Factor w/ 12 levels "May10","May11",..: 12 12
 $ box    : int  4 4 4 4 4 4 4 4 4 4 ...
 $ ISO    : int  2 2 2 2 2 2 2 2 2 2 ...
 $ day.no: int  12 12 11 12 12 12 11 11 12 11 ...
 $ person: Factor w/ 2 levels "A","B": 2 2 1 2 2 2 1 1 2
 $ ccrt   : int  41 52 37 16 33 37 19 45 41 39 ...
```

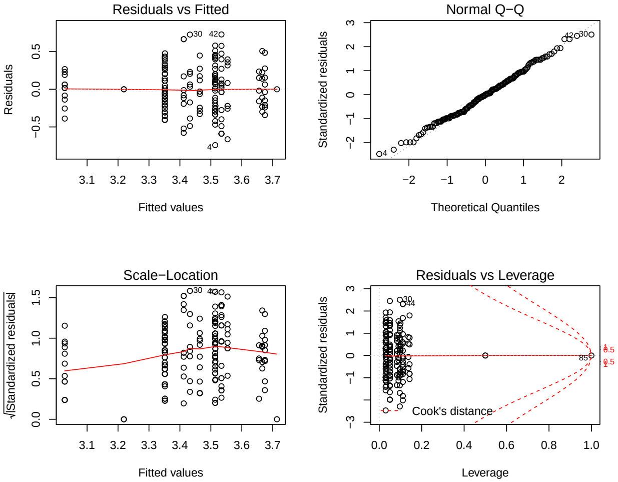
```
> drop1(model,test="F")
Single term deletions

Model:
ccrt ~ line + box + day + person
       Df Sum of Sq   RSS    AIC F value   Pr(F)
<none>                19046 874.41
line    1     58.22 19105 872.97  0.5135 0.47460
box     0      0.00 19046 874.41
day    10   2300.77 21347 875.17  2.0294 0.03318 *
person  1     98.55 19145 873.35  0.8693 0.35250
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```

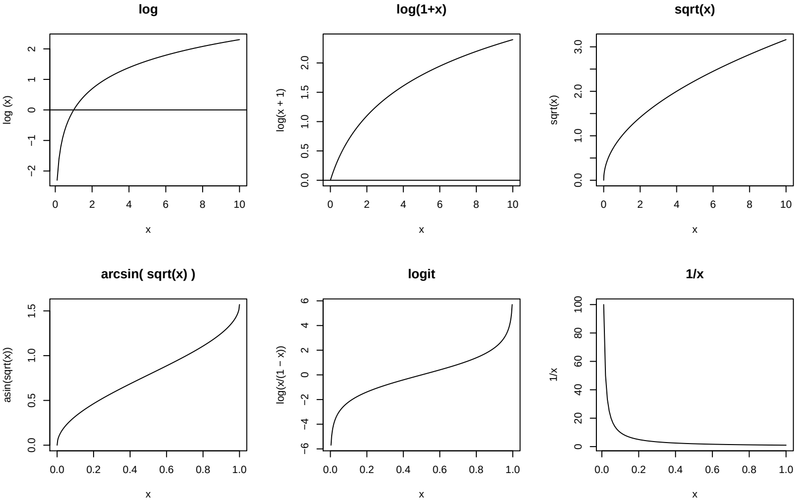

Residuals vs Fitted
Residuals
Fitted values
42
30
44
Normal Q−Q
Standardized residuals
Theoretical Quantiles
Scale−Location
√|Standardized residuals|
Fitted values
Residuals vs Leverage
Standardized residuals
Leverage
Cook's distance
30
67
85
1
0.5

```
> model2 <- lm(log(ccrt)~line+box+day+person,fly)
> drop1(model2,test="F")
Single term deletions

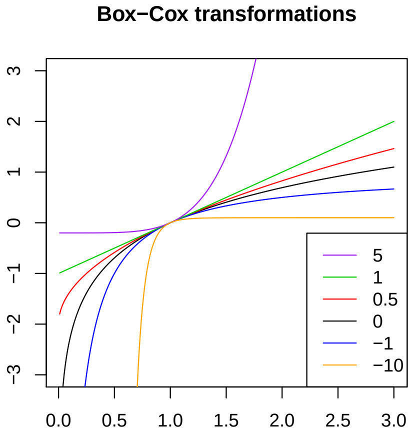
Model:
log(ccrt) ~ line + box + day + person
       Df Sum of Sq    RSS     AIC F value    Pr(F)
<none>                15.618 -418.91
line    1   0.05860 15.677 -420.23  0.6304 0.428338
box     0   0.00000 15.618 -418.91
day    10   2.45864 18.077 -412.30  2.6446 0.005096 **
person  1   0.08504 15.703 -419.92  0.9147 0.340239
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1
```


Residuals vs Fitted
Residuals
Fitted values
Normal Q−Q
Standardized residuals
Theoretical Quantiles
Scale−Location
√|Standardized residuals|
Fitted values
Residuals vs Leverage
Standardized residuals
Leverage
Cook's distance

Popular Transformations



Box-Cox-Transformations



$$f_\lambda(x) = \begin{cases} \log(x) & \text{if} \quad \lambda = 0 \\ \left(x^\lambda - 1\right)/\lambda & \text{if} \quad \lambda \neq 0 \end{cases}$$

```
boxcox(ccrt~line+box+day+person,data=fly)
```