Experimental Design and Data Analysis, Lecture 9

Eduard Belitser

VU Amsterdam

Lecture Overview

1. ANCOVA
2. prediction and feature selection in linear regression:
   - lasso
   - ridge
   - elastic net
3. multiple testing procedures, FDR control

analysis of covariance (ANCOVA)

Setting

An experiment with:

- a numerical outcome Y;
- a factor that can be fixed at I levels.
- a numerical explanatory variable X.

Often the dependence of Y on the numerical variable X is a-priori evident, and the variable is included only to increase the precision of the analysis.

EXAMPLE Experiment to investigate the strength of a wire as dependent on the type of material used and its thickness. (Thickness could not be controlled.)

EXAMPLE Experiment where a subject must press a green or red button if there is a car in the picture shown on the screen, with outcome reaction time, factors presence or not of an auditory stimulus and explanatory variable age of the subject.

Design

- Select NI experimental units randomly from the population of interest.
- Measure the X of each unit.
- Assign level i of the factor randomly to N units.
- Perform the experiment NI times independently.

Randomization is as for one-factor experiments (1-way ANOVA).

The model and hypothesis to test

Data

$$(Y_{i1}, X_{i1}), (Y_{i2}, X_{i2}), \ldots, (Y_{in}, X_{iN}), \quad i = 1, 2, \ldots, I.$$

The linear ANCOVA model assumes that

$$Y_{in} = \mu + \alpha_i + \beta X_{in} + e_{i,n}, \quad i = 1, \ldots, I, \ \ n = 1, \ldots, N,$$

for errors (e_{in}) that can be viewed a random sample from a normal population.

We want test the null hypothesis $H_0 : \alpha_i = 0$, $i = 1, 2, \ldots, I$, and $H_0 : \beta = 0$.

We also want to estimate the parameters $\alpha_1, \ldots, \alpha_I$ and β.

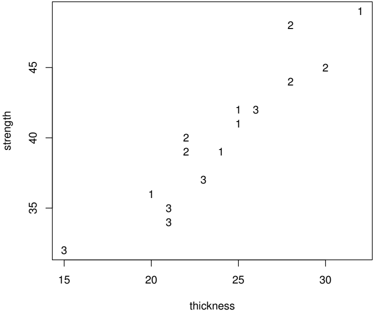
Analysis in R — data input

The data frame contains the data about the strength of a fiber made on 3 different machines. Thickness cannot be controlled, but measured.

```
> fiber=read.table("fiber.txt",header=TRUE); fiber
   strength thickness type
1        36        20    1
2        41        25    1
3        39        24    1
4        42        25    1
5        49        32    1
6        40        22    2
7        48        28    2
8        39        22    2
9        45        30    2
10       44        28    2
11       35        21    3
12       37        23    3
13       42        26    3
14       34        21    3
15       32        15    3
```

Analysis in R — graphics

```
> plot(strength~thickness,pch=as.character(type))
```



Strength clearly increases with thickness. Its dependence on type is not so clear.

Analysis in R — testing (1)

```
> fiber$type=as.factor(fiber$type)
> anova(lm(strength~type,data=fiber))
[ some output deleted ]
          Df Sum Sq Mean Sq F value  Pr(>F)
type       2  140.4  70.200  4.0893 0.04423 *
```

Factor `type` is significant, but one-way ANOVA with only factor `type` is not correct!

```
> fiber1=lm(strength~thickness+type,data=fiber)  # type second!
> anova(fiber1)     # only p-value for type is relevant
[ some output deleted ]
          Df  Sum Sq Mean Sq  F value   Pr(>F)
thickness  1 305.130 305.130 119.9330 2.96e-07 ***
type       2  13.284   6.642   2.6106   0.1181
Residuals 11  27.986   2.544
```

Factor `type` is now insignificant. The output of ANCOVA depends on the order of the variables in the model formula. The correct p-value for `type` is obtained with `strength∼thickness+type`, not with `strength∼type+thickness`. Alternative: use `drop1` instead of `anova`, see next slide.

Analysis in R — testing (2)

```
> drop1(fiber1,test="F")       # here all p-values are relevant
Single term deletions

Model:
strength ~ thickness + type
          Df Sum of Sq     RSS    AIC F value    Pr(>F)
<none>                  27.986 17.355
thickness  1   178.014 206.000 45.297 69.9694 4.264e-06 ***
type       2    13.284  41.270 19.181  2.6106    0.1181
```

The command drop1 is very handy: it performs the tests for the both models, strength∼thickness+type and strength∼type+thickness at once, whereas the *p*-values in the output of anova are sequential, as in a step-up strategy. This problem does not arise in (balanced) ANOVA or linear regression, but it does in an unbalanced ANOVA, ANCOVA and mixed models. Another (and the best) way to get correct p-values, e.g., for the factor type: fiber2=lm(strength∼thickness,data=fiber), then anova(fiber2,fiber1) will give the right p-values for the factor type.

Analysis in R — estimation

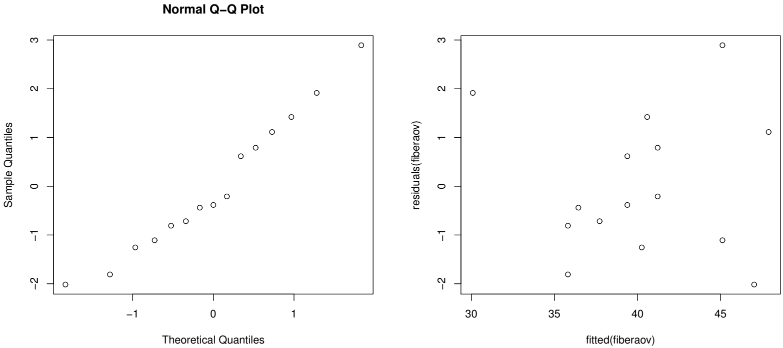
```
> fiber1=lm(strength~thickness+type,data=fiber); summary(fiber1)
[ some output deleted ]
            Estimate Std.Error t value Pr(>|t|)
(Intercept)   17.360   2.961   5.862  0.000109 ***
thickness      0.954   0.114   8.365  4.26e-06 ***
type2          1.037   1.013   1.024  0.328012
type3         -1.584   1.107  -1.431  0.180292
```

This shows the coefficient estimates $\hat{\mu}$, $\hat{\beta}$, $\hat{\alpha}_2$ and $\hat{\alpha}_3$ ($\hat{\alpha}_1 = 0$ as this is the default treatment parameterization). Their confidence intervals can be obtained by `confint(fiber1)`. As $\hat{\beta} = 0.954 > 0$, the thicker the fiber, the stronger it is, the strongest type of fieber is `type2`, although factor `type` is now insignificant. As, in case of anova and linear model, the rest concerns testing the individual hypothesis about the coefficients being zero. For example, the p-value for testing $H_0 : \beta = 0$ (the coefficient for `thickness` variable is 4.26e-06, hence $H_0 : \beta = 0$ is rejected.

Analysis in R — diagnostics

The residuals and fitted values can (and should) be investigated as usual.

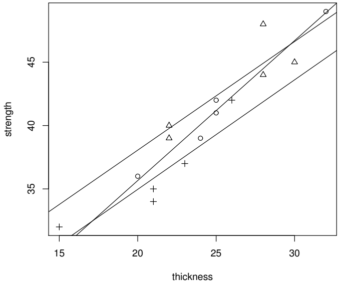
```
> qqnorm(residuals(fiber1))
> plot(fitted(fiber1),residuals(fiber1))
```



Analysis in R – interaction between factor and predictor (1)

The model $Y_{in} = \mu + \alpha_i + \beta X_{in} + e_{in}$ says that within each level i of the factor the dependence of Y on X is a straight line with the same slope.

```
> plot(strength~thickness,pch=unclass(type))
> for (i in 1:3) abline(lm(strength~thickness,data=fiber[fiber$type==i,]))
```



Plot shows no indication that the true lines would not be parallel. We can test for that as follows: fit the model with different slopes $\beta_1, \beta_2, \beta_3$ for each factor level $Y_{in} = \mu + \alpha_i + \beta_i X_{in} + e_{in}$, and then test $H_0 : \beta_1 = \beta_2 = \beta_3$. In other words, this is testing for the interaction between factor type and predictor `thickness`.

Analysis in R – interaction between factor and predictor (2)

Testing for the interaction between factor `type` and predictor `thickness` is done by including the interaction term `type:thickness` in the model.

```
> fiber3=lm(strength~type*thickness,data=fiber); anova(fiber3)
[ some output deleted ]
               Df  Sum Sq Mean Sq F value    Pr(>F)
type            2 140.400  70.200 25.0231 0.0002107 ***
thickness       1 178.014 178.014 63.4538 2.291e-05 ***
type:thickness  2   2.737   1.369  0.4878 0.6292895
Residuals       9  25.249   2.805
```

The model formula is `type*thickness` rather than `type+thickness`. Only the last p-value is relevant which always concerns interaction for models with interaction. We conclude from it that $H_0 : \beta_1 = \beta_2$ is not rejected, i.e., there is no interaction between factor `type` and predictor `thickness`.

Analysis in R – interaction between factor and predictor (3)

```
> summary(fiber3)
[ some output deleted ]
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      13.5722     4.9375   2.749 0.022520 *
type2             7.3421     7.6684   0.957 0.363355
type3             4.1068     6.6631   0.616 0.552932
thickness         1.1043     0.1937   5.702 0.000294 ***
type2:thickness  -0.2471     0.2960  -0.835 0.425337
type3:thickness  -0.2401     0.2843  -0.845 0.420215
```

The estimates of `type2:thickness` and `type3:thickness` give the estimated differences $\hat{\beta}_2 - \hat{\beta}_1$ and $\hat{\beta}_3 - \hat{\beta}_1$. The interaction term is not significant. So, no indication that the initial analysis is in trouble.

Prediction and feature selection in linear regression

Lasso, ridge and elastic net method (1)

- In this case we have only 4 variables to choose from, so we were able to identify the significant ones by a manual inspection of p-values.
- This will quickly become unfeasible if the number of predictors is big.
- An algorithm that could somehow automatically shrink the coefficients of the insignificant variables or (better!) set them to zero altogether?
- This is precisely what lasso and its close cousin, ridge regression, do.
- Lasso and ridge regularization work by adding a penalty term $\lambda P(\beta)$ to the mean residual sum of squares

$$\frac{1}{N}\sum_{n=1}^{N}\big(Y_n-(\beta_0+\beta_1X_{n,1}+\ldots+\beta_pX_{n,p})\big)^2=\frac{\|Y-X\beta\|^2}{N}$$

and minimizing the resulting sum $\frac{1}{N}\|Y-X\beta\|^2+\lambda P(\beta)$ ($2N$ can be used instead of N) with respect to $\beta=(\beta_0,\beta_1,\ldots,\beta_p)\in\mathbb{R}^{p+1}$:

$$\frac{1}{N}\|Y-X\beta\|^2+\lambda P(\beta)\to\min_{\beta}$$

Lasso, ridge and elastic net methods (2)

- Lasso method: $P(\beta) = \|\beta\|_1 = \sum_{k=0}^{p} |\beta_k|$, i.e.,

$$\min_{\beta} \Big\{ \frac{\|Y - X\beta\|^2}{N} + \lambda \|\beta\|_1 \Big\} = \min_{\beta} \Big\{ \frac{\|Y - X\beta\|^2}{N} + \lambda \sum_{k=0}^{p} |\beta_k| \Big\}.$$

- Ridge method: $P(\beta) = \|\beta\|_2^2 = \sum_{k=0}^{p} \beta_k^2$, i.e.,

$$\min_{\beta} \Big\{ \frac{\|Y - X\beta\|^2}{N} + \lambda \|\beta\|_2^2 \Big\} = \min_{\beta} \Big\{ \frac{\|Y - X\beta\|^2}{N} + \lambda \sum_{k=0}^{p} \beta_k^2 \Big\}.$$

- Elastic net method: $P(\beta) = \alpha\|\beta\|_1 + (1-\alpha)\|\beta\|_2^2$ ($0 \leq \alpha \leq 1$ controls the "mix" of ridge and lasso regularisation, with $\alpha = 1$ being "pure" lasso and $\alpha = 0$ being "pure" ridge), i.e.,

$$\min_{\beta} \Big\{ \frac{\|Y - X\beta\|^2}{N} + \lambda\Big(\alpha\|\beta\|_1 + (1-\alpha)\|\beta\|_2^2\Big) \Big\}.$$

- Parameter $\lambda \geq 0$ is a free parameter which is usually selected by using a method called cross-validation.

Lasso, ridge and elastic net methods

- Ridge regression enforces the β coefficients to be lower, but it does not enforce them to be zero. That is, it will not get rid of irrelevant features but rather minimize their impact on the trained model.
- Lasso method overcomes the disadvantage of ridge regression by setting the coefficients β to zero if they are not relevant. One usually ends up with fewer features included in the model than you started with, which is an advantage.
- The R-package glmnet implements the elastic net method (for any $0 \leq \alpha \leq 1$) by R-function glmnet, with particular cases ridge ($\alpha = 0$) and lasso ($\alpha = 1$).
- The choice of λ is done by the cross-validation method, implemented by the R-function cv.glmnet.

Analysis in R: generic code for lasso (ridge and elastic net)

Suppose we have a data frame named data, with its first column being the response variable, and the remaining columns are the features to select from.

```
>library(glmnet)
>x=as.matrix(data[,-1]) #remove the response variable
>y=as.double(as.matrix(data[,1])) #only the response variable
>train=sample(1:nrow(x),0.67*nrow(x)) # train by using 2/3 of the data
>x.train=x[train,]; y.train=y[train]  # data to train
>x.test=x[-train,]; y.test=y[-train] # data to test the prediction quality
>lasso.mod=glmnet(x.train,y.train,alpha=1)
>cv.lasso=cv.glmnet(x.train,y.train,alpha=1,type.measure=’mse’)
>plot(lasso.mod,label=T,xvar="lambda")  #have a look at the lasso path
>plot(cv.lasso) # the best lambda by cross-validation
>plot(cv.lasso$glmnet.fit,xvar="lambda",label=T)
>lambda.min=lasso.cv$lambda.min; lambda.1se=lasso.cv$lambda.1se
>coef(lasso.model,s=lasso.cv$lambda.min) #beta’s for the best lambda
>y.pred=predict(lasso.model,s=lambda.min,newx=x.test) #predict for test
>mse.lasso=mean((y.test-y.pred)^2) #mse for the predicted test rows
```

`lambda.min` is the value of λ that gives minimum mean cross-validated error. The other λ saved is `lambda.1se`, which gives the most regularized model such that error is within one standard error of the minimum.

multiple comparisons

Multiple testing

- H_0 is falsely rejected (type I error) with probability at most α_{ind} $(= 0.05)$.
- Given 2 null hypotheses there are 2 possibilities to make such an error. The probability of at least 1 error is then at most $0.05 + 0.05 = 0.1$.
- Suppose for each of m null hypotheses $H_{0,1}, \dots, H_{0,m}$, the probability of type I error is at most α_{ind}, then the probability of at least 1 error is at most $m\alpha_{ind}$. Indeed,

$$P(\text{at least one } H_{0,i} \text{ is rejected}) \leq \sum_{i=1}^{m} P(H_{0,i} \text{ is rejected}) \leq m\alpha_{ind}.$$

- $P(\text{at least one } H_{0,i} \text{ is rejected})$ is called family-wise error rate (FWER).
- To provide FWER ≤ 0.05, we can impose $\alpha_{ind} \leq \frac{0.05}{m}$ for all $H_{0,i}$. Indeed,

$$\text{FWER} \leq m\alpha_{ind} \leq m\frac{0.05}{m} = 0.05.$$

Multiple testing — Bonferroni correction

- Thus, a simple way to control the family-wise error rate FWER $\leq \alpha_{tot}$ for some overall level α_{tot} is to carry out each individual test with $\alpha_{ind} = \frac{\alpha_{tot}}{m}$, known as the Bonferroni correction.
- This is the same as to compare the individual p-values p_{ind} to $\alpha_{ind} = \frac{\alpha_{tot}}{m}$.
- Adjusted p-values for simultaneous tests p_{adj} are such that if every $H_{0,i}$ with $p_{adj} \leq \alpha_{tot}$ is rejected, then FEWR $\leq \alpha_{tot}$.
- In R, the adjusted p-values are called adjusted P-values for Multiple Comparisons, and are computed by `p.adjust`.
- Adjusted p-value according to Bonferroni correction is $p_{adj} = mp_{ind}$.
- Bonferroni correction is very conservative. Indeed, for reasonable α_{tot} (like 0.05)) and relatively large n (like $n = 100$), there will be very few simultaneously rejected $H_{0,i}$'s, because hardly ever we will have $100p_{ind} \leq 0.05$, or $p_{ind} \leq 0.00005$.

Multiple testing procedures for controlling FWER

Multiple testing arises when:

- there are many parameters of interest.
- investigating all differences $\alpha_i - \alpha_{i'}$ of a set of effects α_i in ANOVA.

The latter is the so called *"a-posteriori testing"*, performed following rejection of a composite hypothesis of the type $H_0 : \alpha_i = 0, i = 1, \ldots, I$.

Bonferroni correction is not the only method to control FWER, alternatives:

- Sidak correction (under indep. assump., slightly better than Bonferroni),
- Holm-Bonferroni method, better than Bonferroni (making it obsolete)
- Hochberg's step-up procedure
- Tukey's procedure (`library(multcomp)`, only for pairwise comparisons).
- some extensions of the above mentioned
- Similarly, one designs simultaneous confidence intervals for a set of parameters that have overall confidence level of $1 - \alpha_{tot}$.

To implement these methods in R: fed with given individual p-values p_{ind} and a specified method, `p.adjust` gives the adjusted p-values p_{adj} (not for Sidak and Tukey's procedures) which should be compared to a specified significance level α_{tot}. The corresponding method rejects those hypothesis for which $p_{adj} \leq \alpha_{tot}$.

Individual p-values obtained in ANOVA

Recall the data `pvc` on the production of the plastic PVC, where 3 operators used 8 different devices called `resin` to produce PVC of size `psize`.

```
> pvc$operator=as.factor(pvc$operator); pvc$resin=as.factor(pvc$resin)
> pvcaov=lm(psize~operator*resin,data=pvc); summary(pvcaov)
[ some output deleted ]
Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       36.2500     0.8598  42.164  < 2e-16 ***
operator2         -0.8500     1.2159  -0.699 0.491216
operator3         -0.9500     1.2159  -0.781 0.442245
resin2            -1.1000     1.2159  -0.905 0.374615
resin3            -5.5500     1.2159  -4.565 0.000126 ***
[ some output deleted ]
resin8             0.5500     1.2159   0.452 0.655078
operator2:resin2   1.0500     1.7195   0.611 0.547175
[ some output deleted ]
operator3:resin8  -2.7000     1.7195  -1.570 0.129454
```

The p-values produced above are not simultaneous. The p-values in the lines `resin2`, `resin3,...` are for testing the individual hypotheses $H_0 : \beta_2 = \beta_1, H_0 : \beta_3 = \beta_1, \ldots$.

Multiple testing in R by Tukey's method

```
> library(multcomp)
> pvcmult=glht(pvcaov,linfct=mcp(resin="Tukey"))
> summary(pvcmult)
          Estimate Std. Error t value Pr(>|t|)
2 - 1 == 0   -1.100      1.216  -0.905   0.9827
3 - 1 == 0   -5.550      1.216  -4.565    <0.01 **
4 - 1 == 0   -6.550      1.216  -5.387    <0.01 ***
5 - 1 == 0   -4.400      1.216  -3.619   0.0251 *
6 - 1 == 0   -6.050      1.216  -4.976    <0.01 ***
7 - 1 == 0   -3.350      1.216  -2.755   0.1538
8 - 1 == 0    0.550      1.216   0.452   0.9998
[ some output deleted ]
8 - 6 == 0    6.600      1.216   5.428    <0.01 ***
8 - 7 == 0    3.900      1.216   3.208   0.0625 .
```

Adjusted *p*-values for simultaneous testing the null hypotheses $H_0 : \beta_2 = \beta_1$, $H_0 : \beta_3 = \beta_1$, $H_0 : \beta_4 = \beta_1$, $\ldots$, $H_0 : \beta_8 = \beta_7$, where β_j is the main effect of the jth level of `resin`. The probability that one or more of these would be less than 0.05 *while* the corresponding null hypothesis were true is less than 0.05. Thus we can "safely" say that *all* differences with p-value < 0.05 are nonzero.

False Discovery Rate (FDR)

- Procedures that control the FWER are considered too conservative for most cases of multiple testing (they lead to a substantial loss in power).
- Beter to control (and less stringent) is the False Discovery Rate (FDR) introduced by Benjamini and Hochberg (1995), the expected proportion of falsely rejected null hypothesis among the rejected hypotheses.
- Possible outcomes when testing m hypotheses simultaneously:

	H_0 is true	H_1 is true	Total
Procedure rejects H_0	V	S	R
Procedure does not reject H_0	U	T	$m - R$
Total	m_0	$m - m_0$	m

V is the number of false positives; T is the number of false negatives.

- $\text{FDR} = E\left(\frac{V}{R}\right)$, where we define $\text{FDR} = 0$ if $R = 0$ (then also $V = 0$).

BH and BY procedures to control FDR

- The Benjamini-Hochberg procedure ensures that its FDR is at most α:
  - Order the p-values $p_{(1)} \leq p_{(2)} \leq \ldots \leq p_{(m)}$ and the null hypotheses $H_{0,(1)}, H_{0,(2)}, \ldots, H_{0,(m)}$ correspondingly;
  - If $k_{max} = \max_k \left(p_{(k)} \leq \frac{\alpha k}{m}\right)$ exists, reject $H_{0,(1)}, \ldots, H_{0,(k_{max})}$; otherwise reject nothing.
- The BH procedure is valid when the m tests are independent.
- The Benjamini-Yekutieli procedure is the generalization of BH procedure (for arbitrary dependence assumptions): instead of m one takes $mc(m)$ where $c(m) = \sum_{i=1}^{m} \frac{1}{i}$, so the BY procedure is a bit more conservative.
- Notice that $k_{max} = \max_k \left(p_{(k)} \leq \frac{\alpha k}{m}\right) = \max_k \left(\frac{mp_{(k)}}{k} \leq \alpha\right)$.
- So, the R command `p.adjust` gives the adjusted ordered p-values $\frac{mp_{(k)}}{k}$, which should be compared to a level α. Similarly for the BY procedure.

Multiple testing in R

```
> p.raw=summary(pvcaov)$coef[,4] # vector of individual (raw) p-values
> p.raw=p.raw[order(p.raw)] # order the p-values
> p.val=as.data.frame(p.raw)
> p.val$Bonferroni=p.adjust(p.val$p.raw,method="bonferroni")
> p.val$Holm=p.adjust(p.val$p.raw,method="holm")
> p.val$Hochberg=p.adjust(p.val$p.raw,method="hochberg")
> p.val$BH=p.adjust(p.val$p.raw,method="BH")
> p.val$BY=p.adjust(p.val$p.raw,method="BY"); round(p.val,3)
                 p.raw Bonferroni  Holm Hochberg    BH    BY
(Intercept)      0.000      0.000 0.000    0.000 0.000 0.000
resin4           0.000      0.000 0.000    0.000 0.000 0.001
resin6           0.000      0.001 0.001    0.001 0.000 0.001
resin3           0.000      0.003 0.003    0.003 0.001 0.003
resin5           0.001      0.033 0.027    0.027 0.007 0.025
resin7           0.011      0.264 0.209    0.209 0.044 0.166
operator3:resin8 0.129      1.000 1.000    0.954 0.444 1.000
operator3:resin5 0.361      1.000 1.000    0.954 0.892 1.000
resin2           0.375      1.000 1.000    0.954 0.892 1.000
operator3        0.442      1.000 1.000    0.954 0.892 1.000
[ some output deleted ]
```

To wrap up

Today we learned:

- ANCOVA
- prediction and feature selection in linear regression
- multiple testing procedures, FDR control

Next time: Logistic regression, Poisson regression