GLS & LME

Lecture 6

LSM3257

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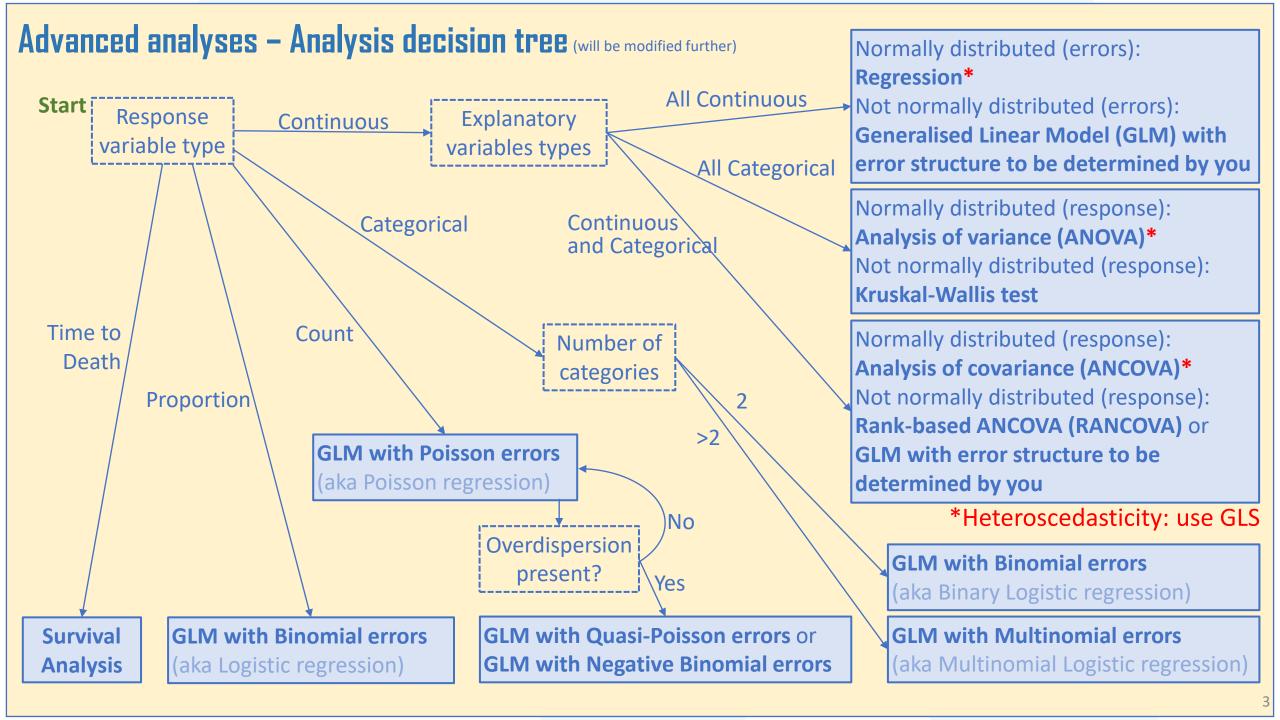
Summary (Learning Objectives)

Generalised Least Squares (GLS)

- Purpose
- Types of variance structures: varIdent, varFixed, varPower, varExp, varConstPower, varComb
- Fitting, checking and comparing

Linear Mixed Effect (LME) models

- Random effects: what are they, why and when to use them?
- Imer() vs. Ime()
- Fitting, checking and comparing
- Simplifying: random effects using REML, then fixed effects using ML





GLS

Generalised Least Squares

What is GLS?

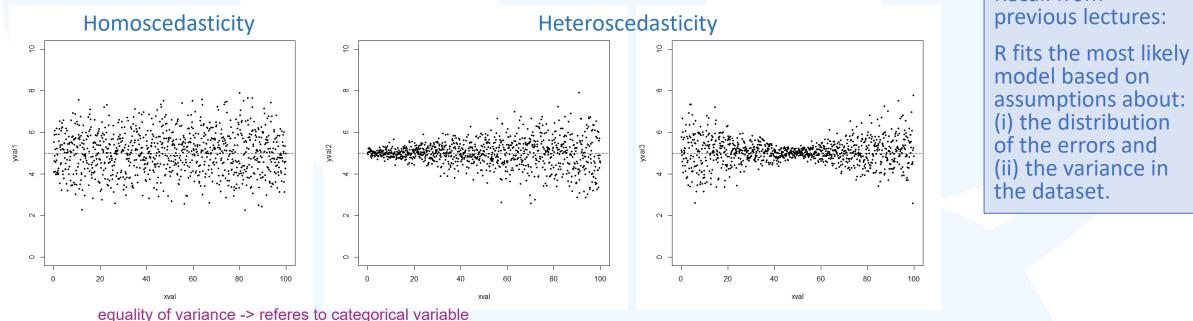
Used if:

one or more explanatory variable

i) You're doing a linear model (i.e. a Regression, ANOVA or ANCOVA; you have one continuous response variable and one or more continuous or categorical response variables); and

ii) Your model has a problem with heteroscedasticity: because variance changes with the values

of a variable, R does not know how to pick the best model.



Recall from previous lectures:

model based on assumptions about: (i) the distribution of the errors and

(ii) the variance in

With GLS, you specify a variance structure (how the variance in the explanatory variable changes) and R will take this into account to fit the best model.

What is GLS?

Types of variance structures you can fit... maybe Jan, Feb, Mar, alll diff

explanatory

Levels of variance (varident): variance changes with the levels of a categorical variable.

Fixed variance (varFixed): variance changes with the value of a continuous covariate (x-variable. Do not use if the covariate has negative values. continuous explanatory variable increase -> variance increase/decrease linear relationship

Power (varPower): variance changes with the power (e.g. x^a) of the covariate. Do not use if the covariate has 0 values.

exponential relationship

Constant+Power (varConstPower): variance changes with the power of the covariate plus a certain constant. Better than varPower if the covariate has values close to 0.

Exponential (varExp): variance changes with the exponent (e.g. a^x) of the covariate. Use this if the covariate has 0 values.

Combination (varComb): used to combine any of the relationships above.

if categorical -> varldent, if continuous, fit all the variance structures and compare

R uses a few parameters (e.g. a) in the above. When you run the GLS, the computer will choose the best value based on maximum likelihood.

Finding variance structure problems in the dataset

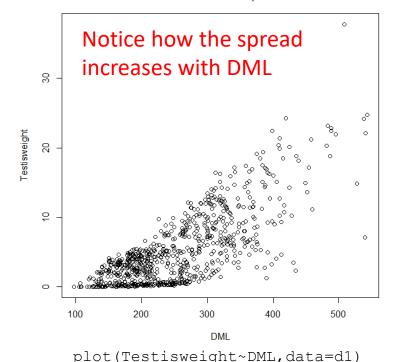
We're using the Squid dataset from <u>Zuur et al.</u> (2009) where they use <MONTH> (categorical) and <DML> (Dorsal Mantle Length; continuous) to explain <Testisweight> (continuous): therefore an ANCOVA.

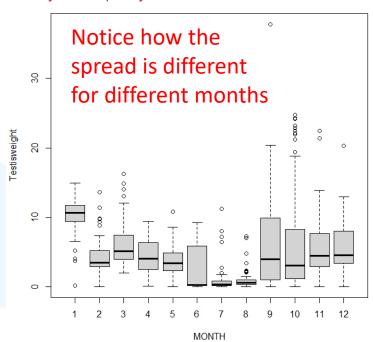
This coplot() is another

#Read in and visualise the dataset

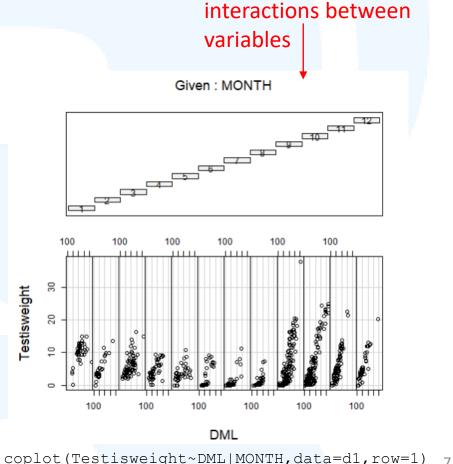
d1=read.table("Squid.txt", header=T)

looks like problem with heteroschadicity and equality of variance





boxplot(Testisweight~MONTH, data=d1)



useful plot to look for

Finding variance structure problems in the dataset

#Install the nlme package to do a GLS

```
require (nlme)
```

#Fit the ANCOVA and check the model

```
mod.lm=gls(Testisweight~DML*MONTH, data=d1)
```

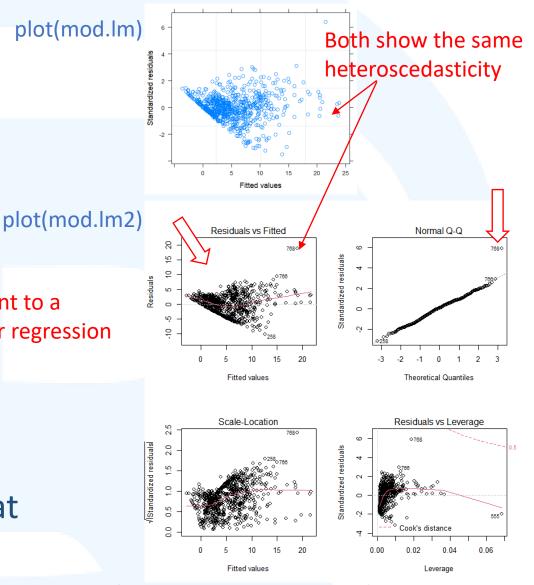
```
plot (mod.lm)
only plot one heteroscedasticity
Without any "weights" specified, this is equivalent to a
linear model, i.e. an ANCOVA (here) or ANOVA or regression
```

```
mod.lm2=lm(Testisweight~DML*MONTH, data=d1)
```

```
par(mfrow=c(2,2))
```

plot (mod.lm2)

#Looks like problems with heteroscedasticity that changes with DML and MONTH



We could try transforming the variable, but we suspect (from the graphs) that variance is changing with the explanatory variables, so we decide to try fitting a GLS to solve the issue.

#Specify variance structure for different levels of <MONTH> (categorical variable)

vs1=varIdent(form=~1|MONTH)

#Fit the GLS

This is the format for specifying a categorical variable

This "weights" command makes this a GLS and tells R how to account for the heteroscedasticity.

mod.ident=gls(Testisweight~DML*MONTH, data=d1, weights=vs1)

#Compare the two models

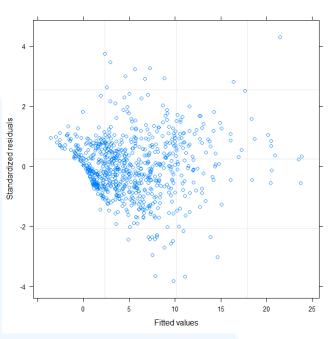
AIC (mod.lm, mod.ident)

#Check results

summary(mod.ident)

```
plot (mod.ident)
```

```
AIC for mod. Ident is
> AIC(mod.lm,mod.ident)
                             lower so it is better!
In AIC.default(mod.lm, mod.ident) :
  models are not all fitted to the same number of observations
Structure: Different standard deviations per stratum
1.3783514 1.6473098 1.4232366 1.9584902 1.9788666
```



The plot suggests there is still more variance structure to account for.

These numbers compare the variance of each month to month 1 (in the boxplots, month 9 is the biggest).

for diffferent months, they are multiplying this number

#Specify variance structure for <DML> (continuous variable)

```
#Fit the GLS If we're using varFixed, we don't need the "form=" and the vertical bar (note: varFixed cannot take categorical variables).
```

mod.fixed=gls(Testisweight~DML*MONTH, data=d1, weights=vs2)

#Compare the models

```
AIC (mod.lm, mod.ident, mod.fixed)
```

#mod.ident is still the best so far

```
> AIC(mod.lm,mod.ident,mod.fixed)

df AIC

mod.lm 25 3641.877

mod.ident 36 3614.436

mod.fixed 25 3620.898

Warning message:
In AIC.default(mod.lm, mod.ident, mod.fixed):

models are not all fitted to the same number of observations
```

#Specify power variance structure for <DML>

vs3a=varPower(form=~DML)

random slope | random intercept

#Specify power variance structure and also allow it to vary with <MONTH>

vs3b=varPower(form=~DML|MONTH)

The left side of the bar usually specifies the slope of a linear equation, and is for continuous variables.

The right side of the bar usually specifies the intercept of a linear equation and is for categorical variables.

Together, this code allows the effect of DML to be different in different months: we're accounting for both a categorical variable and a continuous variable at the same time

#Do the same for constant+power and exponential variance structures for <DML>

```
vs4a=varConstPower(form=~DML)
vs4b=varConstPower(form=~DML|MONTH)
vs5a=varExp(form=~DML)
vs5b=varExp(form=~DML|MONTH)
```

#Fit all the models and compare

```
mod.powA=gls(Testisweight~DML*MONTH, data=d1, weights=vs3a)
mod.powB=gls(Testisweight~DML*MONTH, data=d1, weights=vs3b)
                                                                               df
                                                                                     ATC:
                                                                               36 3614.436
                                                                    mod.ident
mod.conPowA=gls(Testisweight~DML*MONTH, data=d1, weights=vs4a)
                                                                    Mod.powA
                                                                               26 3473.019
                                                                               37 3407.511
mod.conPowB=gls(Testisweight~DML*MONTH, data=d1, weights=vs4b)
                                                                    mod.powB
                                                                    mod.conPowA 27 34751019
mod.expA=gls(Testisweight~DML*MONTH, data=d1, weights=vs5a)
                                                                    mod.conPowB 49 3431.511
                                                                               26 3478 152
                                                                    mod.expA
mod.expB=gls(Testisweight~DML*MONTH, data=d1, weights=vs5b)
                                                                    mod.expB
                                                                               37 3419,719
AIC (mod.ident, mod.powA, mod.powB, mod.conPowA, mod.conPowB, mod.expA, mod.expB)
```

mod.powB looks the best

#Check what mod.powB is doing

```
summary (mod.powB)
```

These are the powers applied to <DML> for each month (e.g. in month 2, the variance is a factor of DML^{1.72}).

#Combining the various functions

```
vs6=varComb (varFixed (~DML), varExp (form=~DML | MONTH))

First structure

Second structure
```

mod.combi=gls(Testisweight~DML*MONTH, data=d1, weights=vs6)

AIC (mod.powB, mod.combi)

> AIC (mod.powB, mod.combi)

df AIC

mod.powB 37 3407.511

mod.combi 37 3415.297

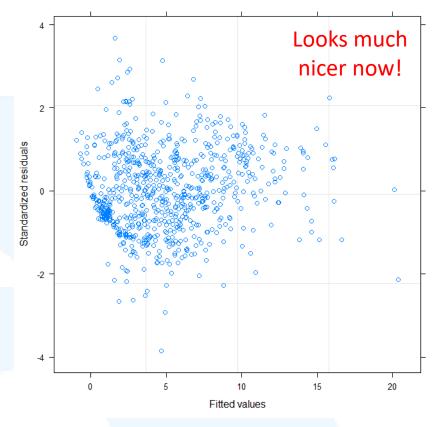
#Check mod.powB

plot (mod.powB)

#Now proceed with model simplification and comparison using AIC()

summary(mod.powB)

#A little harder to read but the information is there, including coefficients and p-values



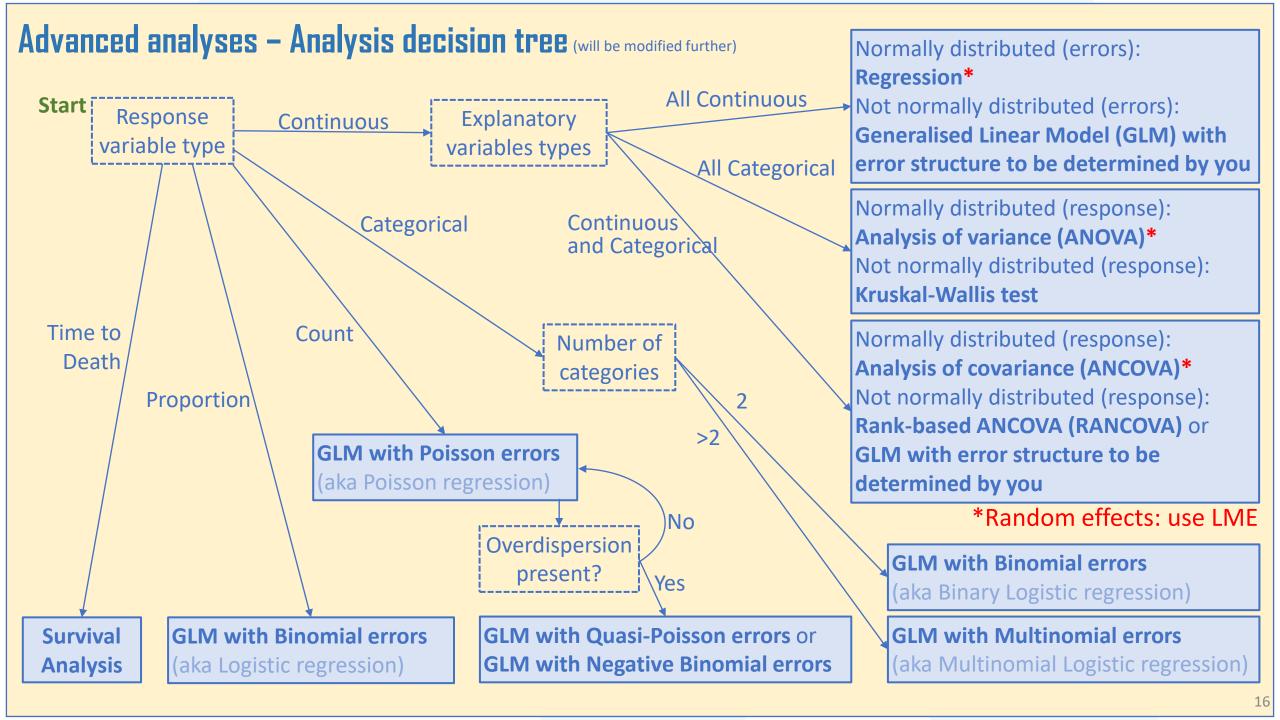
What if GLS doesn't solve my heteroscedasticity problems

Your original normal error distribution assumption may be fatally flawed: therefore do a **Generalised** LM (GLM; in later lectures)



LME

Linear Mixed Effect models



What are Random Effects?

"Random effects" are NOT RANDOM.

So far when we fit a(n) regression/ANOVA/ANCOVA, we partition the variation to:

- 1) the variables we are interested in (the main explanatory variables aka Fixed effects).
- 2) the residuals (Errors due to random chance).
- Additionally, an LME allows us to partition the variation to:
- 3) variables which we expect to have an effect but which we are not interested in for this particular experiment/research question (the **Random effects**).
 - A particular variable can be a Fixed effect in one experiment and a Random effect in another—it all depends on your research question.

Random effects can be categorical ("random intercept") or continuous ("random slope").

Examples of Random Effects

Experiment 1: I measure the effect of shore substrate (Rocky vs. Sandy) on the number of crabs at 10 different beaches.

Response variable: number of crabs.

Explanatory variable (Fixed effect): substrate.

Random effect: 10 beaches.

Beach 1

R S

R S

Beach 9

R S

Beach 10

R S

Beach 7

R S

Beach 8

Experiment 2: I measure the effect of the location of 10 beaches on the number of crabs at the beach, and control for the effects of shore substrate.

Response variable: number of crabs.

Explanatory variable: 10 different beaches.

Random effect: substrate (but not ideal because < 5 levels).

Examples of Random Effects

Experiment 3: I use the **number of people living near a protected area** to explain the **success of the protected area** in **10 different countries** with varying **GDP**.

Response variable: success of protected area.

Explanatory variable: Answer

Random effects: Answer

Experiment 4: I test the effects of butterfly colour (brown and black) and light levels (dim and bright) on mantid attack rates using 25 different mantids.

Response variable: attack rate.

Explanatory variables: Answer

Random effects: Answer

Why include Random Effects?

Accounting for the variation caused by these effects will produce a **better model** of our fixed effects: **more realistic** (a better approximation of what is happening in reality) and **more powerful** (better able to find a true effect).

Why include them as Random effects?

Random effects are estimated differently from fixed effects (Linear unbiased prediction instead of Maximum Likelihood): fewer parameters are estimated, multiple comparisons are avoided and precious Degrees of Freedom are saved.

2 Guidelines for using random effects

- Random effects are important variables that are NOT your research question.
- In general, you want categorical random effects to have at least 5 levels (if the variable has < 5 levels, it is probably better to include it as a fixed effect).

Fitting Linear Mixed Effect models – linear models with random effects

Two functions from two different packages:

1) Ime4 package, Imer() function.

Pros: Can have multiple independent random effects. Faster.

Cons: Cannot specify variance structure. Does not give p-values by default, need to install the ImerTest package as well.

Adding a second random effect

#Example code: mod.lme=lmer($y\sim x1+x2*x3/x4+(1|A/B/C)+(E|D)$) with a random slope "E"

Fixed effects. Can include interactions and nestedness as we have previous learnt

2) nlme package, **lme()** function.

Random effects for variables A, B and C specified like this. C is nested in B is nested in A (e.g. C is leaves, B is trees, A is forest)

Pros: You can also specify variance structure using "weights=" like a GLS.

Cons: Cannot have multiple random intercepts (except if they are nested).

#Example code: mod.lme=lme(y~x1+x2*x3/x4,random=~1|A/B/C)

Fitting LMEs

#Prepare the dataset

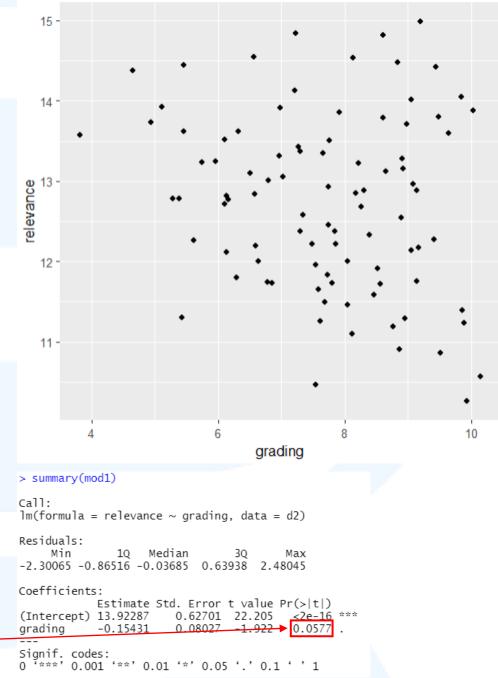
```
d2=read_excel("relevance.xlsx")
d2$origin=as.factor(d2$origin)
d2$process=as.factor(d2$process)
library(ggplot2)
ggplot(data=d2,aes(x=grading,y=relevance))+
geom_point()
```

#Explain <relevance> using <grading>:

#Normal regression

```
mod1=lm(relevance~grading, data=d2)
summary(mod1)
```

Without random effects, the effect of <grading> is marginal



Fitting LMEs – 1 random effect (categorical)

Ime4 package

```
require(lme4)
require(lmerTest) #for p-values
```

Maybe <origin> will help to explain:

```
#1 categorical random effect
mod2a=lmer(relevance~grading+
(1|origin), data=d2)
```

nlme package

require(nlme)

#1 categorical random effect
mod2b=lme(relevance~grading,
random=~1[origin,data=d2)

<origin> is now included as a
random effect in the model. Note
where it is inserted because it is a
categorical variable.

Fitting LMEs - interpreting results

Ime4 package

summary (mod2a)

```
> summarv(mod2a)
Linear mixed model fit by REML. t-tests use
  Satterthwaite's method []merModLmerTest]
Formula: relevance ~ grading + (1 | origin)
REML criterion at convergence: 277.7
Scaled residuals:
              1Q Median
-1.97887 -0.90830 -0.00838 0.63659 2.19413
Random effects:
 Groups
         Name
                     Variance Std.Dev
         (Intercept) 0.1026 0.3203◀
 origin
                     1.1298
                             1.0629
Number of obs: 92, groups: origin, 2
Fixed effects:
                                     df t value
           Estimate Std. Error
(Intercept) 14.19631
                      0.66866 31.22828 21.231
            -0.18990
                       0.08061 89.97977 -2.356
           Pr(>|t|)
            <2e-16 ***
(Intercept)
grading
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Correlation of Fixed Effects:
        (Intr)
grading -0.926
```

require (MuMIn)

r.squaredGLMM(mod2a)

```
> r.squaredGLMM(mod2a)
R2m R2c<
[1,] 0.05556494 0.1342041
```

Variation
explained by
the random
effect

Residual variation

Now significant!

R2m: marginal R², the variation explained by the **fixed effects**

R2c: conditional R², the variation explained by the fixed + random effects

nlme package

summary(mod2b)

```
> summary(mod2b)
Linear mixed-effects model fit by REML
             BIC logLik
  285.7207 295.72 -138.8604
Random effects:
 Formula: ~1 | origin
        (Intercept) Residual
StdDev:
        0.3203465 1.062942
Fixed effects: relevance ~ grading
                Value Std.Error DF t-value p-value
(Intercept) 14.196307 0.6686619 89 21.230920 0.0000
            -0.189904 0.0806112 89 -2.355798 0.0207
 Correlation:
        (Intr)
grading -0.926
Standardized Within-Group Residuals:
-1.978871578 -0.908302014 -0.008382594 0.636584922
 2.194128304
Number of Observations: 92
Number of Groups: 2
```

require (MuMIn)

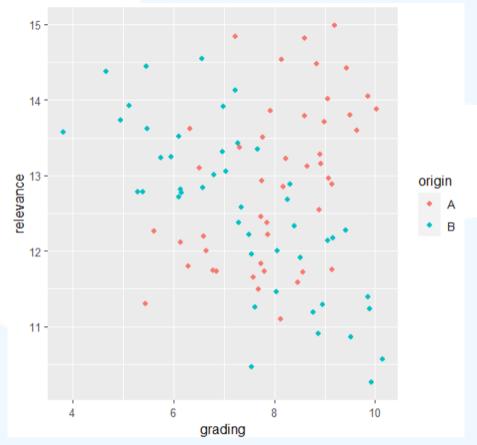
r.squaredGLMM (mod2b)

```
> r.squaredGLMM(mod2b)
R2m R2c
[1,] 0.05556494 0.1342037
```

0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fitting LMEs – interpreting results

Intuitively, adding <origin> as a random effect accounts for the fact that there are two different levels of <origin>: A and B

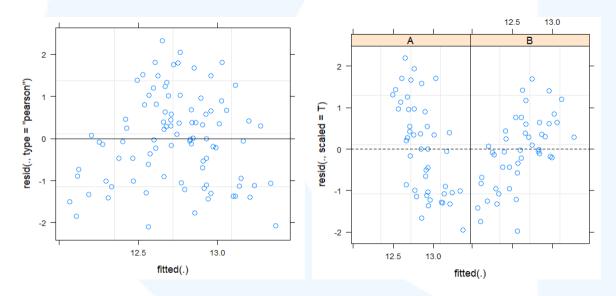


Test your understanding!: if you had fit <origin> as a fixed effect, do you think <grading> and <origin> would have a significant interaction?

Fitting LMEs – diagnostic plots

Ime4 package

#Variance for whole dataset
plot(mod2a)

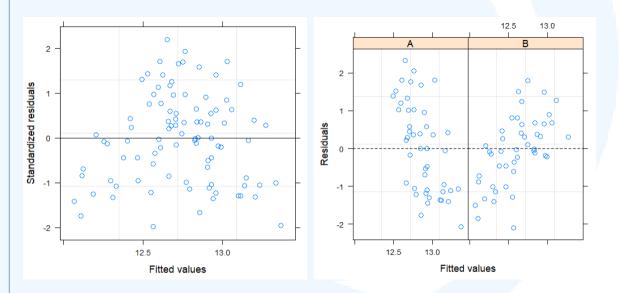


require (lattice)

#Variance for levels of random effect
plot(mod2a, resid(., scaled=T) ~fitted(.)
|origin,abline=0,lty=2)

nlme package

#Variance for whole dataset
plot(mod2b)



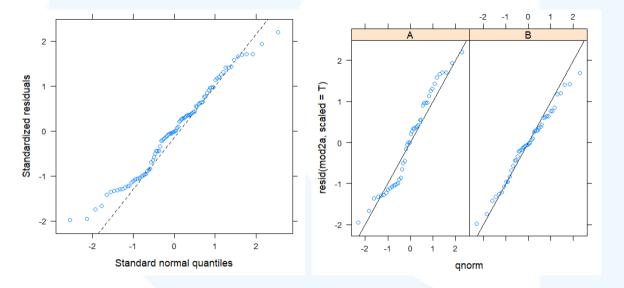
require (lattice)

#Variance for levels of random effect
plot(mod2b, resid(., scaled=T) ~fitted(.)
|origin,abline=0,lty=2)

Fitting LMEs – diagnostic plots

lme4 package

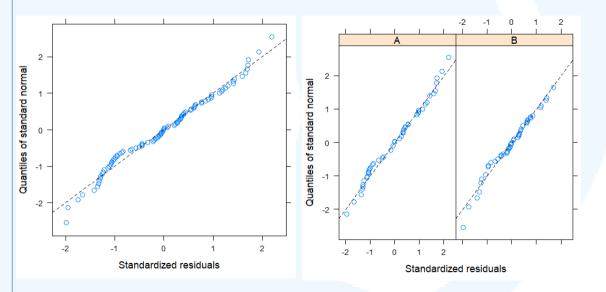
#Normality for whole dataset
qqmath(mod2a,lty=2)



#Normality for levels of random effect
qqmath(~resid(mod2a,scaled=T)|origin,
data=d2,abline=c(0,1))

nlme package

#Normality for whole dataset
qqnorm(mod2b,abline=c(0,1),lty=2)



#Normality for levels of random effect
qqnorm(mod2b,~resid(.,type="p")|origin
,abline=c(0,1),lty=2)

What if heteroscedasticity is present?

Option 1: transform the y-variable.

Option 2: fit variance structures using lme() (using "weights", similar to GLS: just add a "weights=" argument to the model).

Option 3: use GLM.

What if residuals are non-normal?

Option 1: transform the y-variable.

Option 2: use GLM.

Fitting LMEs – 2 nested and 2 separate random effects (categorical)

Ime4 package

#Nested categorical random effects

nlme package

#Nested categorical random effects

```
mod3b=lme(relevance~grading,random=~1|
origin/process,data=d2)
summary(mod3b)
```

#Separate random effects

```
mod4a=lmer(relevance~grading+(1|origin
) *(1|process), data=d2)
```

Just add more variables using "+" or "*" (for interacting random effects, though this is rare)

#Compare the two models

AIC (mod3a, mod4a)

#Separate random effects lme() is not able to do this.

```
> mod3a=lmer(relevance~grading+(1|origin/process),data=d2)
boundary (singular) fit: see ?isSingular ▼
> summary(mod3a)
Random effects:
                            Variance Std.Dev.
 Groups
                Name
 process:origin (Intercept) 0.0000
                                     0.0000
origin
                (Intercept) 0.1026
                                     0.3203
                                     1.0629
                            1.1298
optimizer (nloptwrap) convergence code: 0 (OK)
boundary (singular) fit: see ?isSingular
```

A "boundary fit" doesn't necessarily mean that there's something wrong: here it is because process:origin doesn't explain anything, so you could remove it from the model

Fitting LMEs – 2 random effects (1 continuous and 1 categorical)

Ime4 package

mod5a=lmer(relevance~grading+(time|
origin/process), data=d2)

Continuous variable goes in front of the bar: this adjusts the slope for <time> for the different levels of <origin> and process>

nlme package

mod5b=lme(relevance~grading,random=~time
|origin/process,data=d2)

#Convergence problems: no results/cannot trust results!

> mod5b=lme(relevance~grading,random=~time|origin/process,da
ta=d2) #convergence problems!
Error in lme.formula(relevance ~ grading, random = ~time | o
rigin/process, :
 nlminb problem, convergence error code = 1
 message = iteration limit reached without convergence (10)
In addition: There were 50 or more warnings (use warnings()
to see the first 50)

Convergence problems are frustratingly common in LMEs, GLMs and GLMMs. This usually happens when your **model is too complex** for the package to handle. You CANNOT trust the results! You must adjust your model until there are no more convergence problems.

There are some ways you can handle this issue (I will go through these on another week):

- 1) Fit a simpler model (fewer explanatory variables and/or random effects);
- 2) Adjust some of the settings in the function;
- 3) Try another package;
- 4) Choose a different response variable.

We will take a closer look at this in a later lecture.

Note: continuous random effects are less common than categorical. If you only want to fit one continuous random effect, fit it as a fixed effect.

Simplifying mixed effect models

We simplify the random effects first because we don't want them to explain any of the variation that our fixed effects can explain.

FIRST: simplify random effects—fit models using REML (Restricted Maximum Likelihood which ignores fixed effects) and compare models using AIC().

NEXT: simplify fixed effects—fit models using **ML** (**Maximum Likelihood**) and compare models using either anova() (only if they're subsets) or AIC() (preferred).

Ime4 package

#Fitting models using REML

```
mod3a=lmer(relevance~grading+(1|origin
/process), data=d2, REML=T)
```

REML=T is the default, so you actually don't need to specify this

nlme package

#Fitting models using REML

```
mod3b=lme(relevance~grading,random=~1|
origin/process,data=d2,method="REML")
```

method="REML" is the default, so you actually don't need to specify this

#Fitting models using ML

mod3a=lmer(relevance~grading+(1|origin
/process), data=d2, REML=F)

#Fitting models using ML

mod3b=lme(relevance~grading,random=~1|
origin/process,data=d2,method="ML")

Example – simplify random effects first

Compare models with 3 random effects structures: (i) no random effects (mod6a); (ii) <origin>; and (iii) process> nested within <origin>.

Ime4 package

#Fit models using REML

nlme package

#Fit models using REML

```
mod6a=lm(relevance~grading+time, data=d2)
```

```
mod7a1=lmer(relevance~grading+time+
  (1|origin), data=d2)
mod7a2=lmer(relevance~grading+time+
```

mod7a2=lmer(relevance~grading+time+
(1|origin/process),data=d2)

```
mod7b1=lme(relevance~grading+time,
random=~1|origin,data=d2)
```

mod7b2=lme(relevance~grading+time, random=~1|origin/process,data=d2)

#Test models using AIC()

AIC (mod6a, mod7a1, mod7a2)

#Test models using AIC()

AIC (mod6a, mod7b1, mod7b2)

```
> AIC(mod6a,mod7a1,mod7a2)

df AIC

mod6a 4 281.3751 ←

mod7a1 5 294.9092

mod7a2 6 296.9092
```

In both, the model with no random effects is the best so we could choose the lm() with no random effect structure. But if we-feel that including <origin> is more accurate biologically (i.e. in real life), we can choose to keep it: "the art of data analysis."

Example – simplify fixed effects after

Now we use the random effect structure we chose in the previous step, **fit a new model using "ML"**, and simplify using stepwise deletion.

Ime4 package

#Re-fit using ML

```
mod8a=lmer(relevance~grading+time+(1|
origin),data=d2,REML=F)
summary(mod8a) #<time> can be removed
mod9a=update(mod8a,~.-time)
summary(mod9a) #min. adequate model
```

nlme package

#Re-fit using ML

```
mod8b=lme(relevance~grading+time, rando
m=~1|origin, data=d2, method="ML")
summary(mod8b) #<time> can be removed
mod9b=update(mod8b, ~.-time)
summary(mod9b) #minimum adequate model
```

#Compare using AIC() or anova()

```
AIC (mod8a, mod9a)
```

anova (mod8a, mod9a)

> AIC(mod8a.mod9a)

mod8a 5 282.5272

mod9a 4 281.1875

#Compare using AIC() or anova()

AIC (mod8b, mod9b)

anova (mod8b, mod9b)

> AIC(mod8b,mod9b) df AIC mod8b 5 282.5272 mod9b 4 281.1875

In both cases, the model without <time> is better (i.e. AIC is lower; and no difference in variation explained so we prefer the simpler model). This is our final model.

Summary (Learning Objectives)

Generalised Least Squares (GLS)

- Purpose
- Types of variance structures: varIdent, varFixed, varPower, varExp, varConstPower, varComb
- Fitting, checking and comparing

Linear Mixed Effect (LME) models

- Random effects: what are they, why and when to use them?
- Imer() vs. Ime()
- Fitting, checking and comparing
- Simplifying: random effects using REML, then fixed effects using ML

