

# Dependent data data: mixed effect Models

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From 79 cows the protein content in the milk was measured in the several weeks following calving.

cow  $i$ : the protein content from the first week  $y_{i1}$  and the protein content of the second week  $y_{i2}$ .

These measurements are continuous variables for which the normal distribution can be used. The models to be considered are linear models.

two observations are taken from the same cow which is the sampling unit. These observations are likely to be dependent

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- 1 The first observation contains information about the observation in the second week, since both are from the same cow.
- 2 In the case that for each sampling unit two observations are obtained, the probability distribution must deal with two observations (variables) at the same time. This probability distribution is called a bi-variate probability distribution.

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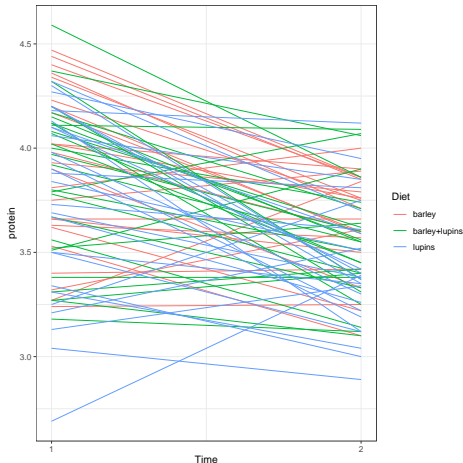


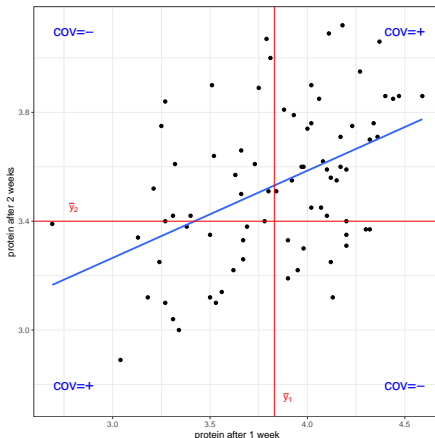
Figure 1: Protein content in the milk of cows in the 2 weeks following calving

# Covariance

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$$\text{cov}(y_1, y_2) = \frac{1}{n-1} \sum_{i=1}^n (y_{i1} - \bar{y}_1)(y_{i2} - \bar{y}_2)$$



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# Correlation

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The size of the covariance has no meaning since it depends on the scale on which the variables are measured.

If for instance a variable is measured in kg and that is changed to gram, then the covariance will change, it is multiplied by 1000. This is a drawback of the covariance.

In order to get a measure that is scale independent, one can divide the covariance by the standard deviations of  $y_1$  and  $y_2$ :

$$r = \frac{\text{cov}(y_1, y_2)}{s_{y_1} s_{y_2}}$$

The correlation between  $y_1$  and  $y_2$  is calculated as 0.46

# t-tests

Compare the independent analysis case to the dependent one.

Variable  $y_1$  is measured from cows from group number 1 and  $y_2$  from cows in group number 2. In that case a two sample t-statistic could be calculated:

$$t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

It is often assumed that the population variances in both groups are the same. Both variances in the denominator are then replaced by  $s^2$ .

The outcome of this t-statistic is 5.4 based on  $78 + 78 - 2 = 154$  degrees of freedom (One cow had missing values). ( $\bar{y}_1 = 3.83$ ,  $\bar{y}_2 = 3.53$ ,  $sd_1 = .401$ ,  $sd_2 = .284$ )

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# t-test

If the data is considered dependent, then the paired t-statistic is calculated.

For each cow the values for  $y_2$  and  $y_1$  are subtracted to get the difference:  $d_i = y_{1i} - y_{2i}$ . The mean of this difference is calculated:

$$\begin{aligned}\bar{d} &= \frac{1}{n} \sum_{i=1}^n d_i \\ &= \frac{1}{n} \sum_{i=1}^n (y_{1i} - y_{2i}) \\ &= \frac{1}{n} \sum_{i=1}^n y_{1i} - \frac{1}{n} \sum_{i=1}^n y_{2i} \\ &= \bar{y}_1 - \bar{y}_2\end{aligned}$$

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# t-tests

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and the paired t-statistic then is :

$$t = \frac{\bar{d}}{\sqrt{\frac{s_d^2}{n}}} = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s_d^2}{n}}}$$

Comparing these t-statistics one can see that the difference is in the denominator, it is in the way the variances are calculated and thus also giving different degrees of freedom.

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In general the formula for calculating the variance of the difference of two dependent variables is :

$$\text{var}(y_1 - y_2) = \text{var}(y_1) + \text{var}(y_2) - 2 \cdot \text{cov}(y_1, y_2)$$

The variances of the differences  $d_i = y_{1i} - y_{2i}$  can be estimated by using the formula above or by calculating the variance of the difference  $s_d^2$  which is 0.37 giving a t-statistic of 7.13 based on 77 degrees of freedom. where  $s_d^2$  is the variance of the differences  $d_i$ .

# conclusion

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So the influence of the dependencies in the data on the data-analysis is shown in the variance: the means and the difference in means are calculated the same in the independent case as in the dependent one but the difference is in the variance.

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- Observations on the same sampling unit are very likely dependent
- This (linear) dependence in the case of continuous data can be measured with the correlation coefficient
- The dependence in the data is influencing the data-analysis through the variance. With dependent data the variances are different as compared to the independent case and thus are the degrees of freedom also different.

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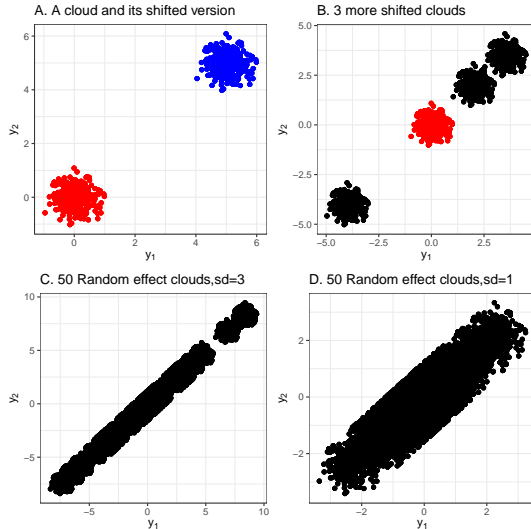


Figure 3: Fixed and random effects

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The coordinates of these shifted clouds have a number in common, one cloud the number 5, an other cloud the number -3 etc. This is the case for the linear models where there are different groups of observations.

All observations within a group have there group mean in common. In that case one can calculate a group effect for every group.

If two variables  $y_1$  and  $y_2$  share a fixed effect then that does not change there independence.



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What does it mean when the outcome of a variable is considered to random?

To get an idea of what random means, think of throwing a die.

Considering the outcome of a variable as random means, that all possible outcomes with there attached probabilities are taken into account .

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Adding a random number to both variables means we have to take all possible outcomes of that variable with there attached probabilities into account and average over it.

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If the same random number is added to two variable, we have to take all possible values of that random number with their attached probability into account, which means we have to take all possible clouds into account and thus have to consider the resulting stretched cloud which implies correlation between the two variables. Or differently, if two variables have a random effect in common, they will be correlated.

If two variables  $y_1$  and  $y_2$  share a random effect then they will be correlated, because then all possible values of the random effect have to be taken into account resulting in a stretched cloud.

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If the standard deviation of the distribution of the random effects is reasonable small as in part D, then these random effects will be concentrated around zero resulting in a condensed cloud.

In that case the correlation between the resulting variables will not be large.

If, however the standard deviation of the distribution of the random effects is reasonable large as in part C. then these random effects will be mostly around zero but large positive or large negative values are also possible resulting in a stretched cloud.

In that case the correlation of the resulting variables will be large.

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The variance of the resulting variable, the variable with the random effects added, will be the variance the original variable had plus the variance of the random effect.

This is how the dependence of the data is modeled, by using random effects and the variance of these random effects determine the height of the correlation.

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There are two observations for each cow.

A linear model could be used for this situation, treating the cow numbers as groups and thus have two observations within each group.

A linear model for this situation might be  $y_{ij} = \mu_i + \epsilon_{ij}$ , where  $\mu_i$  is the mean of cow number  $i$  and  $\epsilon_{ij}$  is the residual, the difference between observation  $y_{ij}$  and the group mean.

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Another formulation of this model is  $y_{ij} = \mu + (\mu_i - \mu) + \epsilon_{ij}$ , where  $\mu$  is the overall mean and  $(\mu_i - \mu)$  is the  $i$  th group (cow) effect.

Using different notation this model can be written as:

$y_{ij} = \beta_0 + b_{0i} + \epsilon_{ij}$ , where  $\beta_0$  is the overall mean,  $b_{0i}$  is the effect of group number  $i$  and  $\beta_0 + b_{0i} = \mu + (\mu_i - \mu) = \mu_i$ , the mean of group  $i$ .

This group effect depends on the overall mean and the group mean, so is the same for each observation  $y_{ij}$  with each group.

Or, to put it different each observation within a group share the same group effect. If this group effect is treated as fixed and we thus have 79 different cow groups, then this does not change the independence.

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If however, the group effects  $b_{0i}$  are random drawings from a normal distribution with mean zero and variance  $\sigma_b^2$ ,

then we have to take all possible values (infinitely many) of that random effect with their attached probabilities into account and as a result we have to consider one big stretched cloud,

instead of many separate clouds in the fixed effect case.

This means that the resulting variables are correlated.



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The random effects have mean zero. So most of the randomly drawn effects will be around zero.

The variance determines the range of possible random effects. If the variance is large then a stretched cloud is obtained, and there will be a high correlation, when the variance is low, the cloud will be more condensed and the correlation will be low.

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The random effect  $b_{0i}$  depends on  $i$  and thus on the cow.

This means that there is one random effect for all the observations on each cow, these random effects having the the same normal distribution.

This is also called a **shared random effects model**.

# More observations per sampling unit

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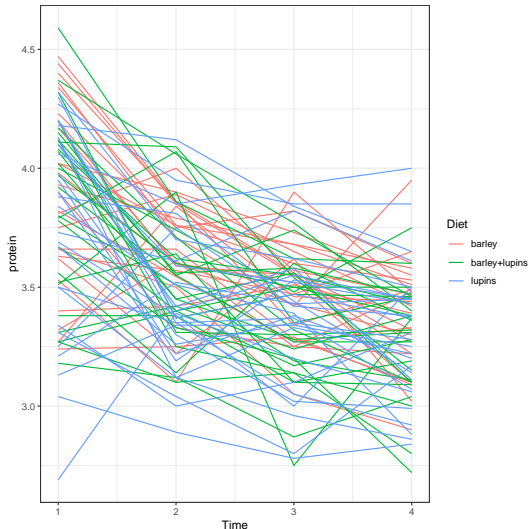


Figure 4: Protein content in the milk of cows in the 4 weeks following

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The anova model is  $y_{ij} = \beta_0 + b_{0i} + \epsilon_{ij}$ , where  $y_{ij}$  is the protein level of cow  $i$  at week  $j$ ,  $\beta_0$  is the overall mean,  $b_{0i}$  is the effect of cow  $i$  and  $\epsilon_{ij}$  is the residual of observation  $y_{ij}$ .

Use the linear model for the case where cow is the grouping variable and there are 4 observations for each cow to write the 4 observations for cow number  $i$  as:

$$\begin{array}{ll} y_{i1} = \beta_0 + b_{0i} + \epsilon_{i1} & y_{i2} = \beta_0 + b_{0i} + \epsilon_{i2} \\ y_{i3} = \beta_0 + b_{0i} + \epsilon_{i3} & y_{i4} = \beta_0 + b_{0i} + \epsilon_{i4} \end{array}$$

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This is the linear model for the one way anova case.

In this case the  $b_{0i}$  are fixed numbers. It represents the deviation for cow  $i$  from the overall mean.

Or, the mean for cow  $i$  is  $\beta_0 + b_{0i}$ , just as in the case of two observations above.

Because the observations for cow  $i$  have a fixed effect in common they are modeled as independent.

Since in that case the cow effects are fixed, conclusions from the study are limited to the cows in the study.

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When the  $b_{0i}$  are treated as being drawn from a  $N(0, \sigma_{int}^2)$ -distribution, this is they are random numbers, then the observations share a random effect and as a consequence, by considering all possible outcomes of the random effect, the observations on the same cow are dependent.

The variance between the cows is  $\sigma_{int}^2$  where int stands for intercept.

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The correlation between any two observations on the same cow is the same, because the drawings are from the same distribution, with the same variance, so the stretching is the same. This correlation structure is called the **exchangeable correlation structure** or **compound symmetry**.

Since the cow effects are random, one can regard the cows in the study as a random sample from a population of cows. This means that the conclusion from this study can be generalized to the population of cows from which the cows in the study were sampled.

The fixed effect part of this model,  $\beta_0$ , is the mean protein level of an average cow. An average cow is a cow with random effect zero.

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# The random intercept model



# The random intercept model

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Let's extend the model with the random effects above to include a linear time effect:

$$y_{ij} = \beta_0 + b_{0i} + \beta_1 \textit{Time} + \epsilon_{ij}$$

Just as in a regression model, time is treated as a continuous variable and  $\beta_1$  is the regression coefficient. In this model there is only one regression coefficient so the decline is modeled the same for every cow. The intercepts, however, are different for every cow.

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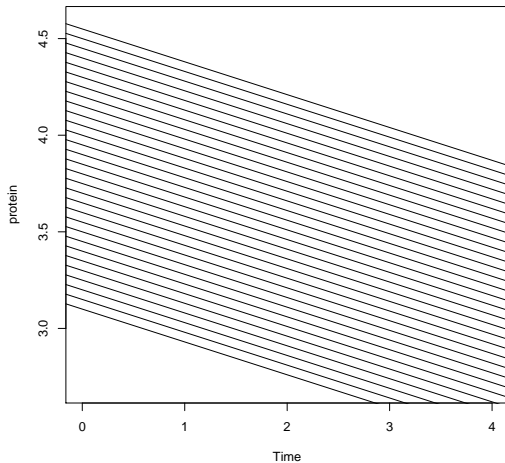


Figure 5: Different intercepts, same slope

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Time is treated as a continuous variable and  $\beta_1$  is the slope.

In this model there is only one regression coefficient so the decline is modeled the same for every cow.

The intercepts, however, are different for every cow.

Recall the model for the analysis of covariance :

$$y_{ij} = \alpha + (\alpha_i - \alpha) + \beta \textit{Time} + \epsilon_{ij}.$$

The general intercept is  $\alpha$  and  $(\alpha_i - \alpha)$  is the effect of group number  $i$  and  $\alpha + (\alpha_i - \alpha) = \alpha_i$  is the mean of group number  $i$ .

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Now replace  $\alpha$  by  $\beta_0$  and  $(\alpha_i - \alpha)$  by  $b_{0i}$  and the model  $y_{ij} = \beta_0 + b_{0i} + \beta_1 \text{Time} + \epsilon_{ij}$  is obtained where the cows are the groups.

In this model the intercept for cow  $i$  is  $\beta_0 + b_{0i}$  where  $\beta_0$  is the general mean of the intercepts and  $b_{0i}$  is the deviation from the general intercept for cow  $i$ .

So this model has different intercepts for the cows and for every cow the same slope. So for every cow different lines are fitted but the lines are parallel.

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If the  $b_{0i}$  are treated as random, then the observations on the same cow have a random number in common and are thus correlated.

The correlation is the same for every pair of observations from the same cow (exchangeable correlation structure).

This model is called **a random intercept model**.

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the model contains a random part and a fixed part:

Random part  $b_{0i} + \epsilon_{ij}$ , the random cow intercepts and the random residuals.

fixed part  $\beta_0 + \beta_1 \text{Time}$ , the general intercept and the regression on time.

One recognizes the fixed part as an ordinary regression model.

This fixed part is a description for the average cow, for a cow with random effect zero.

# The random intercept model

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There were 3 diet-groups: add a grouping variable (2 indicator variables) to the linear part of the model.

- The model contains an intercept and time as a continuous variable. So the fixed part is a regression model.
- One can add the grouping variable diet to the regression model above. The fixed part is then an analysis of covariance model.

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- To the analysis of covariance model the interaction between time and diet can be added. The fixed part is then a model with different intercepts and different slopes.
- Instead of treating time as a continuous variable, one can treat time as a grouping variable. One then has two grouping variables in the model: diet and time. This is a two-way anova model or a two way factorial model.
- In the model with the two grouping variables one can add the interaction between diet and time. This is a full two way factorial model.



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get the data

```
milk <- nlme::Milk[nlme::Milk$Time<3,]
```

For the t test use the function `t.test()` and use `?t.test` for help. \

To make the plot with two time points:

```
library(ggplot2)
ggplot(milk, aes(x=Time, y=protein, group=Cow)) +
  geom_line(aes(color=Diet)) +
  scale_x_continuous(breaks=c(1,2)) +
  theme_bw()
```

```
fit <- lme4::lmer(protein~factor(Time)+
  factor(Diet)+factor(Diet):factor(Time)+
  (1|Cow), REML=FALSE, data = milk)
```

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```
summary(fit,correlation=FALSE)
```

```
## Linear mixed model fit by maximum likelihood ['lmerMod']
## Formula: protein ~ factor(Time) + factor(Diet) + factor(Diet):factor(Time) +
##      (1 | Cow)
##      Data: milk
##
##      AIC      BIC    logLik deviance df.resid
##    106.8    131.3    -45.4     90.8     149
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.87491 -0.56570 -0.03798  0.67019  1.63562
##
## Random effects:
##   Groups   Name                Variance Std.Dev.
##   Cow      (Intercept)  0.04637   0.2153
##   Residual                    0.06784   0.2605
## Number of obs: 157, groups: Cow, 79
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                        3.88680    0.06759  57.506
## factor(Time)2                      -0.24543    0.07474  -3.284
## factor(Diet)barley+lupins          -0.02569    0.09380  -0.274
## factor(Diet)lupins                 -0.12865    0.09380  -1.372
## factor(Time)2:factor(Diet)barley+lupins -0.07568    0.10301  -0.735
## factor(Time)2:factor(Diet)lupins     -0.08494    0.10301  -0.825
```

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# Random coefficients model

# Random coefficients model

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Instead of taking different intercepts for every cow, one could take the slopes different per cow:

$$y_{ij} = \beta_0 + (\beta_1 + b_{1i}) \textit{Time} + \epsilon_{ij} = \beta_0 + \beta_1 \textit{Time} + b_{1i} \textit{Time} + \epsilon_{ij}$$

Now there is only one intercept but every cow has her own slope:  $\beta_1 + b_{1i}$ .

The 4 observations for cow  $i$  can now be described as:

$$\begin{array}{ll} y_{i1} = \beta_0 + (\beta_1 + b_{1i}) \textit{Time} + \epsilon_{i1} & y_{i2} = \beta_0 + (\beta_1 + b_{1i}) \textit{Time} + \epsilon_{i2} \\ y_{i3} = \beta_0 + (\beta_1 + b_{1i}) \textit{Time} + \epsilon_{i3} & y_{i4} = \beta_0 + (\beta_1 + b_{1i}) \textit{Time} + \epsilon_{i4} \end{array}$$

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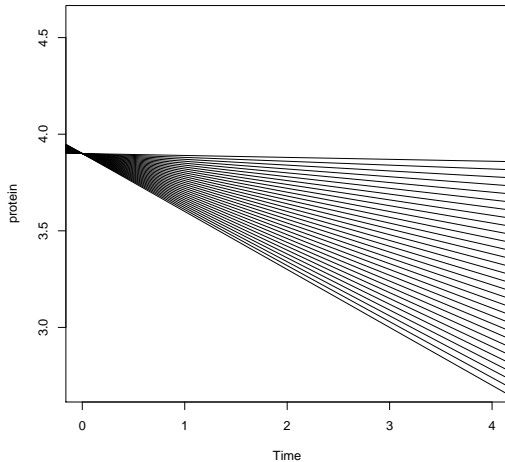


Figure 6: The same intercepts, different slopes

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The  $b_{1i}$  can be treated as random numbers from a  $N(0, \sigma_{slope}^2)$ -distribution,

where  $\sigma_{slope}^2$  is the variance between the slopes of the cows.

In that case the observations from the same cow have a random component in common and thus they are correlated.

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But the random effects  $b_{1i}$  do not get the same weight, as can be seen from the model.

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How heavily  $b_{1i}$  is weighted depends on time. Suppose the time points are 1, 2, 3 and 4. Then:

$y_{i1}$  depends on  $1 \times b_{1i}$

$y_{i2}$  depends on  $2 \times b_{1i}$

$y_{i3}$  depends on  $3 \times b_{1i}$

$y_{i4}$  depends on  $4 \times b_{1i}$

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The  $b_{1i}$  can be treated as random numbers from a  $N(0, \sigma_{slope}^2)$ -distribution, where  $\sigma_{slope}^2$  is the variance between the slopes of the cows.

In that case the observations from the same cow have a random component in common and thus they are correlated.

The weight of the random  $b_{1i}$  on the observation gets larger as the time progresses. This means that the observations on the same cow are correlated but this correlation depends on time.

So, with this model the correlation between  $y_{i1}$  and  $y_{i2}$  and the correlation between  $y_{i1}$  and  $y_{i3}$  are different because the times at which  $y_{i2}$  and  $y_{i3}$  are measured are different.

Usually the correlation between two observations decreases if they are further apart in time.

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So with the random part one models the correlation structure: if only random intercepts are taken the correlation between the observations is constant (exchangeable), with random coefficients the correlations depend on time.

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Here the model also consists of a random and a fixed part:

Random part  $b_{1i} Time + \epsilon_{ij}$ , the random cow slopes and the random residuals.

Fixed part  $\beta_0 + \beta_1 Time$ , the general intercept and the regression on time.

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In a mixed effect model the random part models the correlation structure and the fixed part models the patterns in the data.

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# Model with random intercepts and random coefficients

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A model with a different intercept and different slope for each cow is:

$$y_{ij} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time} + \epsilon_{ij} = (\beta_0 + b_{0i}) + \beta_1 \text{Time} + b_{1i} \text{Time} + \epsilon_{ij}$$

Now every cow has her own intercept  $\beta_0 + b_{0i}$  and her own slope:  $\beta_1 + b_{1i}$ . The 4 observations for cow  $i$  can now be described as:

$$y_{i1} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time} + \epsilon_{i1}$$

$$y_{i2} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time} + \epsilon_{i2}$$

$$y_{i3} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time} + \epsilon_{i3}$$

$$y_{i4} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time} + \epsilon_{i4}$$

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The  $b_{0i}$  are treated as a draw from a  $N(0, \sigma_{int}^2)$ -distribution, where  $\sigma_{int}^2$  is the variance between the intercepts per cow.

The  $b_{1i}$  can be treated as a random number from a  $N(0, \sigma_{slope}^2)$ -distribution, where  $\sigma_{slope}^2$  is the variance between the slopes of the cows.

In that case the observations from the same cow have two random components in common, one of which is weighted by time.

The observations from the same cow are thus correlated and the correlation structure again depends on time.

The correlation structure in this model is more complex as compared to the random coefficient model.

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Again the model consists of a random and a fixed part:

Random part  $b_{0i} + b_{1i} Time + \epsilon_{ij}$ , the random cow intercepts and slopes and the random residuals.

fixed part  $\beta_0 + \beta_1 Time$ , the general intercept and the regression on time.

The fixed part in this model is again just a regression model. Also here a linear model with diet can be used.

And time can be taken as a grouping variable and interactions can be added, just as with the other models.

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In a mixed effect model the random part models the correlation structure and the fixed part models the patterns in the data.



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## Fitting the models

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Write down the likelihood for each model discussed above, and maximize this likelihood with respect to all the parameters in the model in order to obtain the maximum likelihood estimates.

If these estimates are plugged into the likelihood then the maximum value of the likelihood is obtained for the model used.

One can then fit another model (by leaving out one of the terms) and obtain the maximum of the likelihood for that model.

Then these two models can be compared using the AIC or the likelihood ratio test.

This is just the standard likelihood procedure. This procedure works fine for the fixed effect part.

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Using the standard likelihood procedure here gives estimates for the different variance components that can be biased. For this reason the likelihood procedure is modified.

The likelihood is transformed in such a way that the transformed likelihood does no longer depend on the fixed part of the model.

Attention is restricted to the random effects part.

For that reason one calls this the restricted likelihood. This restricted likelihood is maximized to obtain the maximum likelihood estimates, now called restricted maximum likelihood estimates.

This procedure is known as restricted maximum likelihood estimation: REML

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Because the transformation used in REML depends on the fixed part of the model, a change in this fixed part gives another transformation and thus also a different random part.

If one then compares the models not only the change in the fixed part is measured but also a change in the random part. For this reason REML is not appropriate for testing the fixed part of the model.

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A reasonable procedure is to first fit the complete model and test the random part using REML.

After that, refit this model using maximum likelihood and test the fixed part. This procedure is illustrated below.

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```
mlk <- nlme::Milk[nlme::Milk$Time<5,]  
fit <- lme4::lmer(protein~factor(Time)+factor(Diet)+  
  factor(Diet):factor(Time)+(1+Time|Cow),data = mlk)
```

The fixed part of the model is stated in the usual way.

The random part of the model is the part between brackets:  
(1+Time|Cow). The part 1+Time shows the random part of the  
model.

The |Cow part indicates that the random effects are per cow, so cow is  
treated as factor. So the 1 in this model represents the random cow  
intercepts.

This is a model with random (cow) intercepts and random (cow)  
slopes.

Note that the 1 in (1+Time|Cow) can be left out so (Time|Cow)  
represents the same model.

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# Fitting the models

```
summary(fit,correlation=FALSE )
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: protein ~ factor(Time) + factor(Diet) + factor(Diet):factor(Time) +
##      (1 + Time | Cow)
##      Data: mlk
##
## REML criterion at convergence: 90.6
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.67360 -0.53186  0.03346  0.46926  2.39156
##
## Random effects:
##   Groups   Name                Variance Std.Dev. Corr
##   Cow      (Intercept)  0.134877  0.36726
##           Time          0.007439  0.08625  -0.87
##   Residual                0.039697  0.19924
## Number of obs: 315, groups: Cow, 79
##
## Fixed effects:
##                                     Estimate Std. Error t value
## (Intercept)                        3.886800   0.071183  54.603
## factor(Time)2                      -0.247943   0.059672  -4.155
## factor(Time)3                      -0.388800   0.066076  -5.884
## factor(Time)4                      -0.510400   0.076511  -6.671
## factor(Diet)barley+lupins          -0.025689   0.098786  -0.260
## factor(Diet)lupins                 -0.128652   0.098786  -1.302
## factor(Time)2:factor(Diet)barley+lupins -0.073169   0.082321  -0.889
## factor(Time)3:factor(Diet)barley+lupins -0.126756   0.091699  -1.382
## factor(Time)4:factor(Diet)barley+lupins -0.072933   0.106180  -0.687
## factor(Time)2:factor(Diet)lupins     -0.082428   0.082321  -1.001
```

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From the part Random effects it can be seen that the standard deviation between the intercepts is 0.367 (variance is 0.135) and between the slopes 0.086.

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The correlation between these two estimates is  $-0.87$ .

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This means that the two random effects are not modeled as independent. The random intercepts and the random slope have a bi-variate distribution instead of each having an independent normal distribution.

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So this is a model with correlated random intercept and random slope. This is default in the lme4 library.

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To specify a model in which the random intercept and slope are not correlated one can use  $(1+Time||Cow)$ .

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Sometimes one finds that the correlation between the estimate for the random intercept and slope is  $-1$ .

This means that they cannot be distinguished. In that case the model cannot be estimated.

A remedy for this is to change the time scale in the random effects, by dividing it by an appropriate number. If that does not work one can try to fit a model with only random slopes or only random intercepts.

# Fitting the models

correlation matrix:

corr.	time 1	time 2	time 3	time4
time 1	1.000000	0.612828	0.475646	0.274812
time 2	0.612828	1.000000	0.489545	0.344990
time 3	0.475646	0.489545	1.000000	0.412471
time 4	0.274811	0.344990	0.412471	1.000000

As can be seen the correlation between the protein values at time 1 and time 2 is 0.61, between times 1 and 3 it is 0.48 and between times 1 and 4 0.27.

This illustrates something seen more often in time series: the further away the time points are, the lower their correlation.

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The model is fitted using the REML procedure by default (REML=TRUE).

This is used to determine what model for the random part is best according to the data.

Next two other models are fitted with the same fixed effects but with different random effects.

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```
fit2 <- lme4::lmer(protein~factor(Time)+factor(Diet)+  
  factor(Diet):factor(Time)+(1|Cow),data = mlk)  
fit3 <- lme4::lmer(protein~factor(Time)+factor(Diet)+  
  factor(Diet):factor(Time)+(-1+Time|Cow),  
  data = mlk)  
AIC(fit,fit2,fit3)  
  
##      df      AIC  
## fit   16 122.5821  
## fit2  14 138.3831  
## fit3  14 182.8353
```

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Now the fixed effects can be tested, beginning with the interaction term. But first the model has to be fitted again using the maximum likelihood method instead of REML. After that a `drop1()` command is used to see if the interaction is needed:

```
fitml <- lme4::lmer(protein~factor(Time)+factor(Diet)+  
  factor(Diet):factor(Time)+(1+Time|Cow),  
  REML=FALSE, data = mlk)  
  
drop1(fitml)  
  
## Single term deletions  
##  
## Model:  
## protein ~ factor(Time) + factor(Diet) + factor(Diet):factor(Time) +  
##      (1 + Time | Cow)  
##  
##               Df      AIC  
## <none>                73.170  
## factor(Time):factor(Diet)  6 66.792
```

# Fitting the models

The model where the interaction is deleted has the lowest AIC so we can remove the interaction. Let's fit that model:

```
fitml2 <- lme4::lmer(protein~factor(Time)+factor(Diet)+  
  (1+Time|Cow), REML=FALSE, data = mlk)  
  
drop1(fitml2)
```

```
## Single term deletions  
##  
## Model:  
## protein ~ factor(Time) + factor(Diet) + (1 + Time | Cow)  
##           Df      AIC  
## <none>          66.792  
## factor(Time)  3 166.985  
## factor(Diet)  2  67.739
```

There is not much difference between the first and third AIC's so we prefer the simpler model, that is the model with Diet deleted, so only Time in it. There is not enough evidence from the data that the diets are different w.r.t. the protein level. One can use `confint()` to obtain profile likelihood confidence intervals.

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Suppose that, in the cow example, at every time point it was measured whether or not the protein level was high.

One then measures binary data. This data should be modeled with logistic regression.

To deal with the dependencies in the data one can add a random intercept and random slopes to the linear part of the model, just as with an ordinary linear model.

That is, with a logistic regression model the dependencies are modeled on the logit scale.



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The logistic model becomes:

$$\ln \left( \frac{\pi}{1 - \pi} \right) = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) \text{Time}$$

$\beta_0$  are the general log-odds at Time 0, and  $b_{0i}$  are the deviations from these general log-odds for every cow.

These  $b_{0i}$  are taken as draws from a normal distribution. In this way the data are modeled as being dependent, but on the logit scale.  $\beta_1$  is the general coefficient for time and the  $b_{1i}$  are the deviations from this general time effect, now taken as random draws.

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The glmer function from the lme4 library is now used. To fit for example a logistic regression model with random intercepts, and with diet and time as grouping variables plus their interaction for the fixed part, one uses:

```
mlk$highprotein <- 1*(mlk$protein>3.5) #arbitrary choice
fith <- lme4::glmer(highprotein~factor(Time)+factor(Diet)+
  factor(Diet):factor(Time)+(1|Cow),family=binomial,
  data=mlk)
```

# Logistic regression with random effects

```
summary(fith,correlation=FALSE)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
## Family: binomial ( logit )
## Formula:
## highprotein ~ factor(Time) + factor(Diet) + factor(Diet):factor(Time) +
##   (1 | Cow)
## Data: mlk
##
##           AIC          BIC    logLik deviance df.resid
##      339.8       388.6    -156.9     313.8       302
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.7141 -0.4008 -0.0865  0.3727  2.8154
##
## Random effects:
##   Groups Name      Variance Std.Dev.
##   Cow      (Intercept) 4.261    2.064
## Number of obs: 315, groups: Cow, 79
##
## Fixed effects:
##
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)      2.65965    0.83435   3.188  0.00143
## factor(Time)2     -0.90172    0.89179  -1.011  0.31195
## factor(Time)3     -2.22688    0.87507  -2.545  0.01093
## factor(Time)4     -4.21687    0.99792  -4.226 2.38e-05
## factor(Diet)barley+lupins -0.18307    1.11129  -0.165  0.86915
## factor(Diet)lupins -1.38568    1.05503  -1.313  0.18905
## factor(Time)2:factor(Diet)barley+lupins -1.47117    1.23299  -1.193  0.23280
## factor(Time)3:factor(Diet)barley+lupins -1.76819    1.26317  -1.400  0.16157
```

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```
drop1(fith)
```

```
## Single term deletions
```

```
##
```

```
## Model:
```

```
## highprotein ~ factor(Time) + factor(Diet) + factor(Diet):factor(Time) +
```

```
##      (1 | Cow)
```

```
##                                     Df      AIC
```

```
## <none>                             339.83
```

```
## factor(Time):factor(Diet)  6 333.06
```

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```
fith2 <- lme4::glmer(highprotein~factor(Time)+  
  factor(Diet):factor(Time)+(1|Cow),  
  family=binomial,data=mlk)
```

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Fixed effects:	Estimate	Std.Error	z value
(Intercept)	2.58488	0.83157	3.108
factor(Time)2	-0.83389	0.89061	-0.936
factor(Time)3	-2.20941	0.87305	-2.531
factor(Time)4	-4.21328	0.99950	-4.215
factor(Time)1:factor(Diet)barley+lupins	-0.02166	1.12297	-0.019
factor(Time)2:factor(Diet)barley+lupins	-1.61567	1.00723	-1.604
factor(Time)3:factor(Diet)barley+lupins	-1.87950	0.99343	-1.892
factor(Time)4:factor(Diet)barley+lupins	-2.30493	1.23267	-1.870
factor(Time)1:factor(Diet)lupins	-1.25853	1.05970	-1.188
factor(Time)2:factor(Diet)lupins	-2.61526	1.03328	-2.531
factor(Time)3:factor(Diet)lupins	-1.49477	0.97779	-1.529
factor(Time)4:factor(Diet)lupins	-1.35072	1.12815	-1.197