Progressive motility

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Introduction

The effect of DMSO and ethanol is evaluated at concentrations from 0 up to 2% on spermatozoa progressive motility.

DMSO

```
mp_dmso <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "progressive motility_DMSO")
mp_dmso$donor <- as.factor(mp_dmso$donor)
names(mp_dmso) <- c("donor", "conc", "prog", "total")
mp_dmso$prog_frac <- mp_dmso$prog / mp_dmso$total
skimr::skim(mp_dmso)
```

Table 1: Data summary

Name	mp_dmso
Number of rows	40
Number of columns	5
_____	
Column type frequency:	
factor	1
numeric	4
_____	
Group variables	None

Variable type: factor

skim_variable	n_missing	complete_rate	ordered	n_unique	top_counts
donor	0	1	FALSE	8	1: 5, 2: 5, 3: 5, 4: 5

Variable type: numeric

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100	hist
conc	0	1	0.72	0.74	0.0	0.10	0.50	1.00	2.00	
prog	0	1	88.30	42.36	33.0	62.75	77.50	102.25	249.00	
total	0	1	131.20	56.45	54.0	92.00	124.50	150.50	301.00	
prog_frac	0	1	0.67	0.10	0.4	0.62	0.69	0.74	0.86	

There are eight donors, no missing data.

```
table(mp_dmso$donor, as.factor(mp_dmso$conc))
```

```
  0 0.1 0.5 1 2
1 1   1   1 1 1
2 1   1   1 1 1
3 1   1   1 1 1
4 1   1   1 1 1
5 1   1   1 1 1
6 1   1   1 1 1
7 1   1   1 1 1
8 1   1   1 1 1
```

The data are balanced with one observation for each concentration and each donor and no missing data.

```
mp_dmso_m1 <- glmer(cbind(prog, total - prog) ~ conc + (conc | donor),
  data = mp_dmso, family = binomial(link = "logit"))
summary(mp_dmso_m1)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(prog, total - prog) ~ conc + (conc | donor)
```

```
   Data: mp_dmso

     AIC      BIC   logLik deviance df.resid
   339.9    348.3   -164.9    329.9       35

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.8987 -0.9856 -0.2334  0.7372  6.0252

Random effects:
 Groups Name        Variance Std.Dev. Corr
 donor  (Intercept) 0.10858  0.3295
        conc        0.01235  0.1111   -0.16
Number of obs: 40, groups:  donor, 8

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.91013    0.12473   7.297 2.95e-13 ***
conc        -0.21091    0.05805  -3.633  0.00028 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.285
```

The model is:

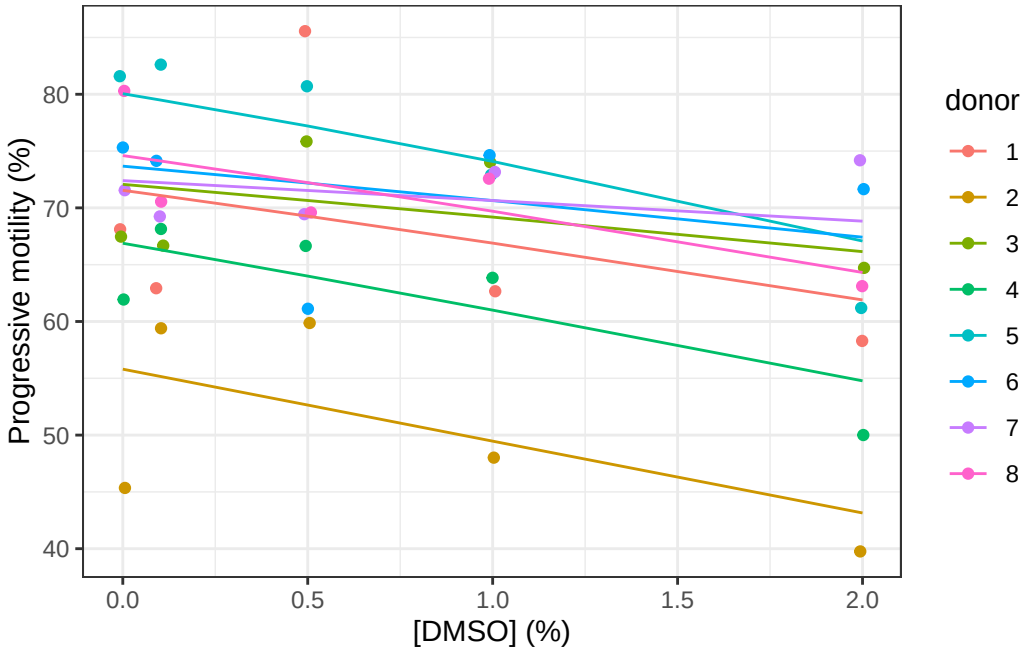
,

$$
\begin{aligned}
\text{prog}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{prog}=1} = \widehat{P}) \\
, \log\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_{1j[i]}(\text{conc}) \qquad , \qquad (1) \\
\begin{pmatrix} \alpha_j \\ \beta_{1j} \end{pmatrix} &\sim N\left(\begin{pmatrix} \mu_{\alpha_j} \\ \mu_{\beta_{1j}} \end{pmatrix}, \begin{pmatrix} \sigma^2_{\alpha_j} & \rho_{\alpha_j\beta_{1j}} \\ \rho_{\beta_{1j}\alpha_j} & \sigma^2_{\beta_{1j}} \end{pmatrix}\right), \text{for donor j} = 1, \ldots, \text{J}
\end{aligned}
$$

Here is a plot of this model:

```
ggplot(data = mp_dmso) +
  geom_jitter(aes(x = conc, y = prog_frac * 100, col = donor),
    width = 0.01) +
```

```
  geom_line(aes(x = conc, y = fitted(mp_dmso_m1) * 100, col = donor)) +
  labs(x = "[DMSO] (%)", y = "Progressive motility (%)")
```



Here, we have all negative slopes. Data are rather widespread. There are definitely shifts in the intercept per donor (different motility at [DMSO] = 0). Slopes seems different between donors. There is an extreme point for donor 1, DMSO 0.5%. Let's check if the model can be simplified with a likelihood ratio test:

```
mp_dmso_m2 <- glmer(cbind(prog, total - prog) ~ conc  + (1 | donor),
  data = mp_dmso, family = binomial(link = "logit"))
anova(mp_dmso_m1, mp_dmso_m2, refit = FALSE) # Despite the name, it is indeed a LR test
```

```
Data: mp_dmso
Models:
mp_dmso_m2: cbind(prog, total - prog) ~ conc + (1 | donor)
mp_dmso_m1: cbind(prog, total - prog) ~ conc + (conc | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
mp_dmso_m2    3 337.14 342.21 -165.57   331.14
mp_dmso_m1    5 339.87 348.32 -164.94   329.87 1.2721  2     0.5294
```

The likelihood ratio does not detect significant differences between the full and simplified models at $\alpha = 5\%$. We could use the simplest `mp_dmso_m2` model with only a shift in the slope per donor.

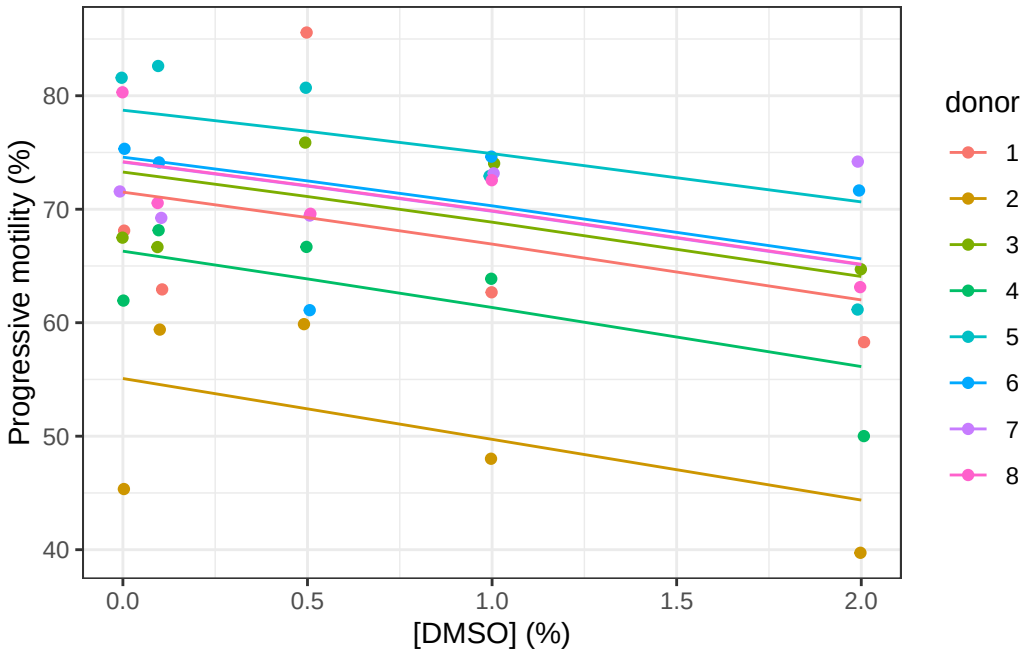
This model is:

,

$$\begin{aligned} \text{prog}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{prog}=1} = \widehat{P}) \\ \text{, log}\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_1(\text{conc}) \qquad \text{,} \\ \alpha_j &\sim N\left(\mu_{\alpha_j}, \sigma^2_{\alpha_j}\right), \text{ for donor j} = 1, \ldots, \text{J} \end{aligned} \tag{2}$$

Here is a plot of this model:

```
ggplot(data = mp_dmso) +
  geom_jitter(aes(x = conc, y = prog_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(mp_dmso_m2) * 100, col = donor)) +
  labs(x = "[DMSO] (%)", y = "Progressive motility (%)")
```



```r
summary(mp_dmso_m2)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(prog, total - prog) ~ conc + (1 | donor)
   Data: mp_dmso

     AIC      BIC   logLik deviance df.resid
   337.1    342.2   -165.6    331.1       37

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.8853 -1.0519 -0.2145  0.8927  6.0275

Random effects:
 Groups Name        Variance Std.Dev.
 donor  (Intercept) 0.1069   0.3269
Number of obs: 40, groups:  donor, 8

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.91430    0.12360   7.397 1.39e-13 ***
conc        -0.21504    0.04105  -5.239 1.62e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.240
```

The Z test indicates that `conc` is significantly different from zero at $\alpha = 5\%$. However, it is not the best test in the case of a mixed model like here. We prefer to rely on the 95% confidence interval calculated either on the profile, or via parametric bootstrap (and especially the later one):

```r
confint(mp_dmso_m2, level = 0.95) # 95% CI based on profile
```

```
Computing profile confidence intervals ...
```

```
                 2.5 %     97.5 %
.sig01       0.2049673  0.6001027
(Intercept)  0.6450188  1.1870224
conc        -0.2954498 -0.1344784
```

```r
set.seed(964)
# 1000x parameter bootstrap
(mp_dmso_m2_conf <- confint(mp_dmso_m2, level = 0.95, method = "boot", nsim  = 1000L))
```

```
Computing bootstrap confidence intervals ...
```

```
3 message(s): boundary (singular) fit: see help('isSingular')
```

```
                 2.5 %     97.5 %
.sig01       0.1276551  0.4774600
(Intercept)  0.6812327  1.1625425
conc        -0.3043773 -0.1325507
```

Among the 1000 bootstrapped models, two are singular. We can ignore this warning, since this impact is probably negligible on the overall calculations. Slope for `conc` is significantly different from zero at $\alpha = 5\%$ because the 95% CI does not contain zero.

Additional verifications

We could double-check the significance of the slope `conc` by looking at a likelihood ratio test when dropping `conc` from the model:

```r
#drop1(mp_dmso_m2, scope = "conc")
mp_dmso_m3 <- glmer(cbind(prog, total - prog) ~ 1 + (1 | donor),
  data = mp_dmso, family = binomial(link = "logit"))
anova(mp_dmso_m2, mp_dmso_m3, refit = TRUE)
```

```
Data: mp_dmso
Models:
mp_dmso_m3: cbind(prog, total - prog) ~ 1 + (1 | donor)
mp_dmso_m2: cbind(prog, total - prog) ~ conc + (1 | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
```

```
mp_dmso_m3    2 362.36 365.74 -179.18   358.36
mp_dmso_m2    3 337.14 342.21 -165.57   331.14 27.221  1  1.814e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The model with `conc` is significantly different at α level 5% from a reference model that sets the slope `conc` $= 0$. There is thus a significant effect of DMSO concentration (confirmation of results obtained from 95% CI).

We also double-check convergence of the model by trying different optimisation engines (just to make sure). First, is there a singularity in the model?

```
isSingular(mp_dmso_m2)
```

```
[1] FALSE
```

… then, a report about the model convergence:

```
mp_dmso_m2_all <- allFit(mp_dmso_m2)
```

```
Loading required namespace: dfoptim
```

```
Loading required namespace: optimx
```

```
bobyqa : [OK]
Nelder_Mead : [OK]
nlminbwrap : [OK]
nmkbw : [OK]
optimx.L-BFGS-B : [OK]
nloptwrap.NLOPT_LN_NELDERMEAD : [OK]
nloptwrap.NLOPT_LN_BOBYQA : [OK]
```

```
summary(mp_dmso_m2_all)
```

```
$which.OK
                        bobyqa                     Nelder_Mead
                          TRUE                            TRUE
                    nlminbwrap                           nmkbw
                          TRUE                            TRUE
              optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                          TRUE                            TRUE
    nloptwrap.NLOPT_LN_BOBYQA
                          TRUE

$msgs
$msgs$bobyqa
NULL

$msgs$Nelder_Mead
NULL

$msgs$nlminbwrap
NULL

$msgs$nmkbw
NULL

$msgs$`optimx.L-BFGS-B`
NULL

$msgs$nloptwrap.NLOPT_LN_NELDERMEAD
NULL

$msgs$nloptwrap.NLOPT_LN_BOBYQA
NULL


$fixef
                               (Intercept)       conc
bobyqa                           0.9142878 -0.2150449
Nelder_Mead                      0.9142827 -0.2150454
nlminbwrap                       0.9142878 -0.2150458
nmkbw                            0.9144157 -0.2150565
optimx.L-BFGS-B                  0.9142877 -0.2150448
nloptwrap.NLOPT_LN_NELDERMEAD    0.9143021 -0.2150591
nloptwrap.NLOPT_LN_BOBYQA        0.9142875 -0.2150433
```

```
$llik
                      bobyqa                     Nelder_Mead
                    -165.5716                     -165.5716
                   nlminbwrap                         nmkbw
                    -165.5716                     -165.5716
              optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                    -165.5716                     -165.5716
    nloptwrap.NLOPT_LN_BOBYQA
                    -165.5716

$sdcor
                              donor.(Intercept)
bobyqa                                0.3269477
Nelder_Mead                           0.3269484
nlminbwrap                            0.3269484
nmkbw                                 0.3268936
optimx.L-BFGS-B                       0.3269497
nloptwrap.NLOPT_LN_NELDERMEAD         0.3269479
nloptwrap.NLOPT_LN_BOBYQA             0.3269549

$theta
                              donor.(Intercept)
bobyqa                                0.3269477
Nelder_Mead                           0.3269484
nlminbwrap                            0.3269484
nmkbw                                 0.3268936
optimx.L-BFGS-B                       0.3269497
nloptwrap.NLOPT_LN_NELDERMEAD         0.3269479
nloptwrap.NLOPT_LN_BOBYQA             0.3269549

$times
                              user.self sys.self elapsed user.child sys.child
bobyqa                            0.046    0.000   0.046          0         0
Nelder_Mead                       0.058    0.000   0.057          0         0
nlminbwrap                        0.051    0.001   0.054          0         0
nmkbw                             0.058    0.000   0.059          0         0
optimx.L-BFGS-B                   0.346    0.002   0.349          0         0
nloptwrap.NLOPT_LN_NELDERMEAD     0.057    0.000   0.059          0         0
nloptwrap.NLOPT_LN_BOBYQA         0.040    0.000   0.041          0         0

$feval
                      bobyqa                     Nelder_Mead
                          70                              87
```

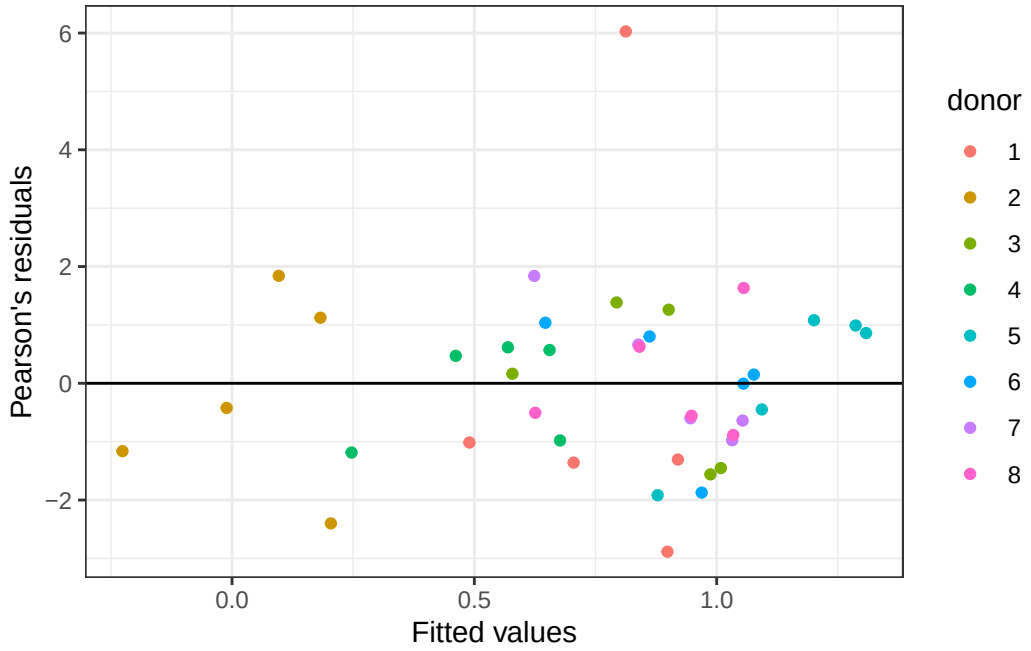
```
                    nlminbwrap                              nmkbw
                            NA                                 96
              optimx.L-BFGS-B  nloptwrap.NLOPT_LN_NELDERMEAD
                            12                                 97
    nloptwrap.NLOPT_LN_BOBYQA
                            30
```

```
attr(,"class")
[1] "summary.allFit"
```

Analysis of the residuals

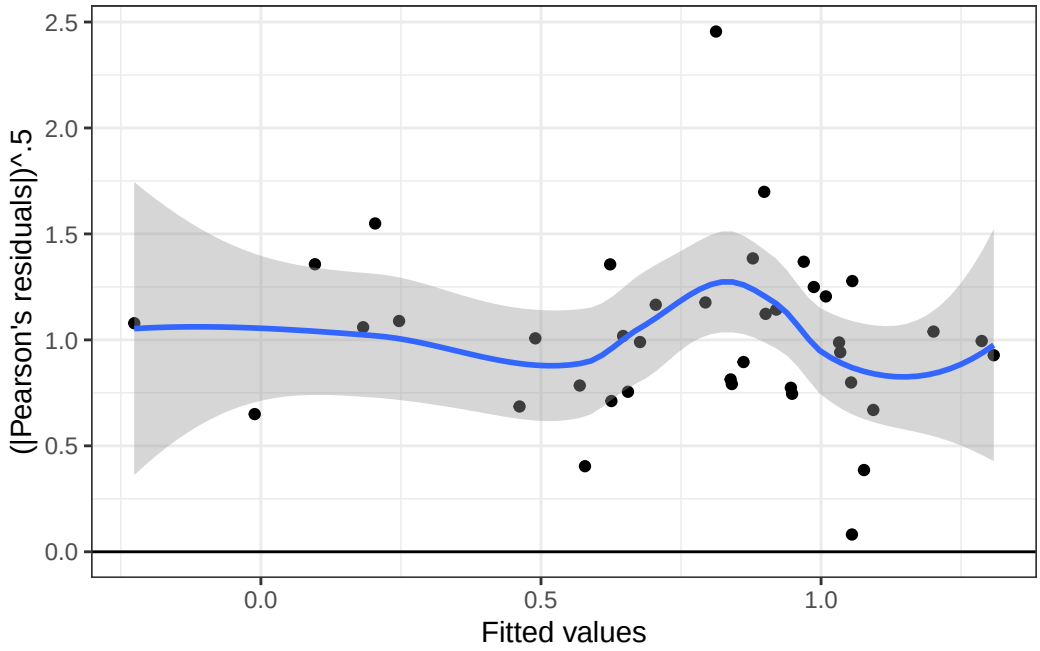
Let's check how the residuals distribute and if there is homoscedasticity.

```
mp_dmso <- fortify.merMod(mp_dmso_m2)
ggplot(data = mp_dmso, aes(x = .fitted, y = .scresid, col = donor)) +
  geom_point() +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "Pearson's residuals")
```



There is one extreme value, but otherwise, residuals are correctly distributed. Linearity is good here.

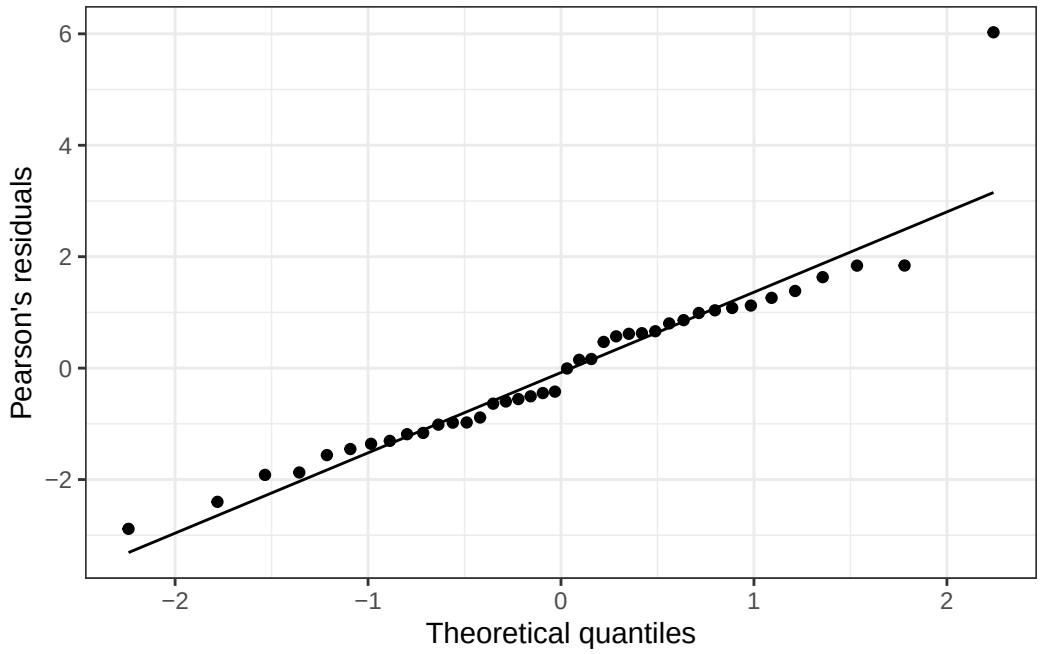
```
ggplot(data = mp_dmso, aes(x = .fitted, y = sqrt(abs(.scresid)))) +
  geom_point() +
  geom_smooth(method = "loess", formula = y ~ x) +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "(|Pearson's residuals|)^.5")
```



Homoscedasticity of the residuals seems here acceptable (the blue curve that is a loess smoothing in the data is relatively horizontal). Of course, the extreme value impacts the loess curve locally, but otherwise the variance in the residuals is homogeneous along fitted values.

Note: according to Gauss-Markov theorem that indicates that linearity, random sample, non-collinearity between predictors, non-correlation between predictors and error term and homoscedasticity are the only requirements for our GLMM, we do not have to check it. Also the model is robust to departure of Normality, and none of the tests we made depend on a Normal distribution of the residuals (we said we don't trust z/t tests and replace them by likelihood ratio tests and parameterized bootstrapped confidence intervals). However, for completeness, here is the quantile-quantile plot of the residuals:

```
ggplot(data = mp_dmso, aes(sample = .scresid)) +
  geom_qq() +
  geom_qq_line() +
  labs(x = "Theoretical quantiles", y = "Pearson's residuals")
```



It appears to be excellent, except for our extreme value, of course. A Shapiro-Wilk test does not confirm Normality, but we are pretty sure it is caused by the extreme value when looking at the quantile-quantile plot:

```
shapiro.test(mp_dmso$.scresid)
```

```
    Shapiro-Wilk normality test

data:  mp_dmso$.scresid
W = 0.90587, p-value = 0.002846
```

Predictions

The model allows to calculate the drop in progressive mobility according to DMSO concentration from 0 to 2%. Note that an inverse logit transformation is required. Here is an example:

```
mp_dmso_slope <- c(
  ci95_min   = min(mp_dmso_m2_conf["conc", ]),
  estimate = fixef(mp_dmso_m2)[["conc"]],
  ci95_max  = max(mp_dmso_m2_conf["conc", ]))
```

```
mp_dmso_slope
```

```
  ci95_min   estimate   ci95_max
-0.3043773 -0.2150449 -0.1325507
```

```
#saveRDS(mp_dmso_slope, "../data/motility_prog_DMSO_slope.rds")
```

Let's say we want to calculate the drop in progressive mobility for various DMSO concentrations between 0 and 2% if the progressive mobility of a sample without DMSO is 80%. The calculation is:

```
predict_logit <- function(conc, intercept = 1, slopes) {
  slopes_mat <- matrix(slopes, nrow = 1,
    dimnames = list(NULL, names(slopes)))
  data.frame(conc = conc, -intercept +
      boot::inv.logit(boot::logit(intercept) +
      conc %*% slopes_mat))
}
dmso_conc <- (0:20) / 10
mp_dmso_lost <- predict_logit(dmso_conc, 0.8, mp_dmso_slope)
mp_dmso_lost
```

```
   conc     ci95_min     estimate     ci95_max
1   0.0  0.000000000  0.000000000  0.000000000
2   0.1 -0.004914533 -0.003462926 -0.002129247
3   0.2 -0.009918142 -0.006970298 -0.004275373
4   0.3 -0.015010889 -0.010522152 -0.006438391
5   0.4 -0.020192755 -0.014118503 -0.008618308
6   0.5 -0.025463637 -0.017759344 -0.010815128
7   0.6 -0.030823344 -0.021444650 -0.013028855
8   0.7 -0.036271596 -0.025174371 -0.015259487
9   0.8 -0.041808018 -0.028948437 -0.017507021
10  0.9 -0.047432141 -0.032766757 -0.019771449
11  1.0 -0.053143399 -0.036629214 -0.022052762
12  1.1 -0.058941127 -0.040535669 -0.024350946
13  1.2 -0.064824556 -0.044485961 -0.026665986
14  1.3 -0.070792815 -0.048479903 -0.028997861
15  1.4 -0.076844928 -0.052517282 -0.031346550
16  1.5 -0.082979813 -0.056597864 -0.033712025
```

```
17  1.6 -0.089196280 -0.060721386 -0.036094258
18  1.7 -0.095493030 -0.064887562 -0.038493216
19  1.8 -0.101868656 -0.069096079 -0.040908863
20  1.9 -0.108321642 -0.073346597 -0.043341158
21  2.0 -0.114850360 -0.077638750 -0.045790059
```

```
#saveRDS(mp_dmso_lost, "../data/motility_prog_DMSO_lost.rds")
```

This is the lost in progressive mobility that the model predicts.

Ethanol

```
mp_etoh <- readxl::read_excel("../data/Table S2.xlsx",
  sheet = "progressive motility_EtOH")
mp_etoh$donor <- as.factor(mp_etoh$donor)
names(mp_etoh) <- c("donor", "conc", "prog", "total")
mp_etoh$prog_frac <- mp_etoh$prog / mp_etoh$total
skimr::skim(mp_etoh)
```

Table 4: Data summary

Name	mp_etoh
Number of rows	40
Number of columns	5
_____	
Column type frequency:	
factor	1
numeric	4
_____	
Group variables	None

Variable type: factor

skim_variable	n_missing	complete_rate	ordered	n_unique	top_counts
donor	0	1	FALSE	8	1: 5, 2: 5, 3: 5, 4: 5

Variable type: numeric

skim_variable	n_missing	complete_rate	mean	sd	p0	p25	p50	p75	p100	hist
conc	0	1	0.72	0.74	0.00	0.10	0.50	1.00	2.00	
prog	0	1	79.80	41.47	30.00	49.75	70.50	90.25	211.00	
total	0	1	127.20	56.93	66.00	87.00	117.00	140.25	301.00	
prog_frac	0	1	0.63	0.14	0.27	0.53	0.68	0.72	0.82	

There are also the same eight donors, no missing data.

```
table(mp_etoh$donor, as.factor(mp_etoh$conc))
```

```
  0 0.1 0.5 1 2
1 1   1   1 1 1
2 1   1   1 1 1
3 1   1   1 1 1
4 1   1   1 1 1
5 1   1   1 1 1
6 1   1   1 1 1
7 1   1   1 1 1
8 1   1   1 1 1
```

The data are balanced with one observation for each concentration and each donor and no missing data.

```
mp_etoh_m1 <- glmer(cbind(prog, total - prog) ~ conc + (conc | donor),
  data = mp_etoh, family = binomial(link = "logit"))
summary(mp_etoh_m1)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(prog, total - prog) ~ conc + (conc | donor)
   Data: mp_etoh

     AIC      BIC   logLik deviance df.resid
   344.5    352.9   -167.2    334.5       35

Scaled residuals:
    Min      1Q  Median      3Q     Max
```

```
-4.2577 -0.8537  0.1724  0.9715  2.1316

Random effects:
 Groups Name        Variance Std.Dev. Corr
 donor  (Intercept) 0.192212 0.43842
        conc        0.006819 0.08258  0.00
Number of obs: 40, groups:  donor, 8

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.80299    0.16168   4.967 6.81e-07 ***
conc        -0.34557    0.05287  -6.536 6.31e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.172
```

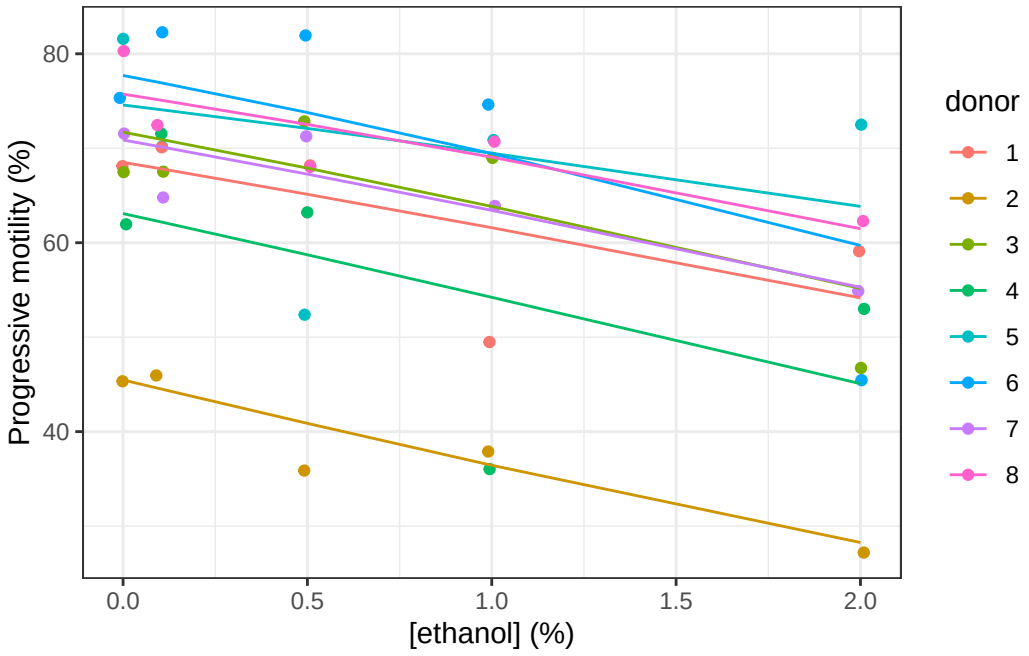
The model is:

,

$$
\begin{aligned}
\text{prog}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{prog}=1} = \widehat{P}) \\
\log\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_{1j[i]}(\text{conc}) \\
\begin{pmatrix} \alpha_j \\ \beta_{1j} \end{pmatrix} &\sim N\left(\begin{pmatrix} \mu_{\alpha_j} \\ \mu_{\beta_{1j}} \end{pmatrix}, \begin{pmatrix} \sigma^2_{\alpha_j} & \rho_{\alpha_j\beta_{1j}} \\ \rho_{\beta_{1j}\alpha_j} & \sigma^2_{\beta_{1j}} \end{pmatrix}\right), \text{for donor j} = 1, \ldots, \text{J}
\end{aligned}
\quad , \qquad (3)
$$

Here is a plot of this model:

```
ggplot(data = mp_etoh) +
  geom_jitter(aes(x = conc, y = prog_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(mp_etoh_m1) * 100, col = donor)) +
  labs(x = "[ethanol] (%)", y = "Progressive motility (%)")
```



Slopes are all negative, suggesting a negative concentration effect. Data are rather widespread. There are definitely shifts in the intercept per donor (different motility at [etoh] = 0). Slopes seems very similar between donors. Let's check if the model can be simplified using a likelihood ratio test:

```
mp_etoh_m2 <- glmer(cbind(prog, total - prog) ~ conc  + (1 | donor),
  data = mp_etoh, family = binomial(link = "logit"))
anova(mp_etoh_m1, mp_etoh_m2, refit = FALSE) # Despite the name, it is indeed a LR test
```

```
Data: mp_etoh
Models:
mp_etoh_m2: cbind(prog, total - prog) ~ conc + (1 | donor)
mp_etoh_m1: cbind(prog, total - prog) ~ conc + (conc | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
mp_etoh_m2    3 341.03 346.09 -167.51   335.03
mp_etoh_m1    5 344.46 352.91 -167.23   334.46 0.5634  2     0.7545
```

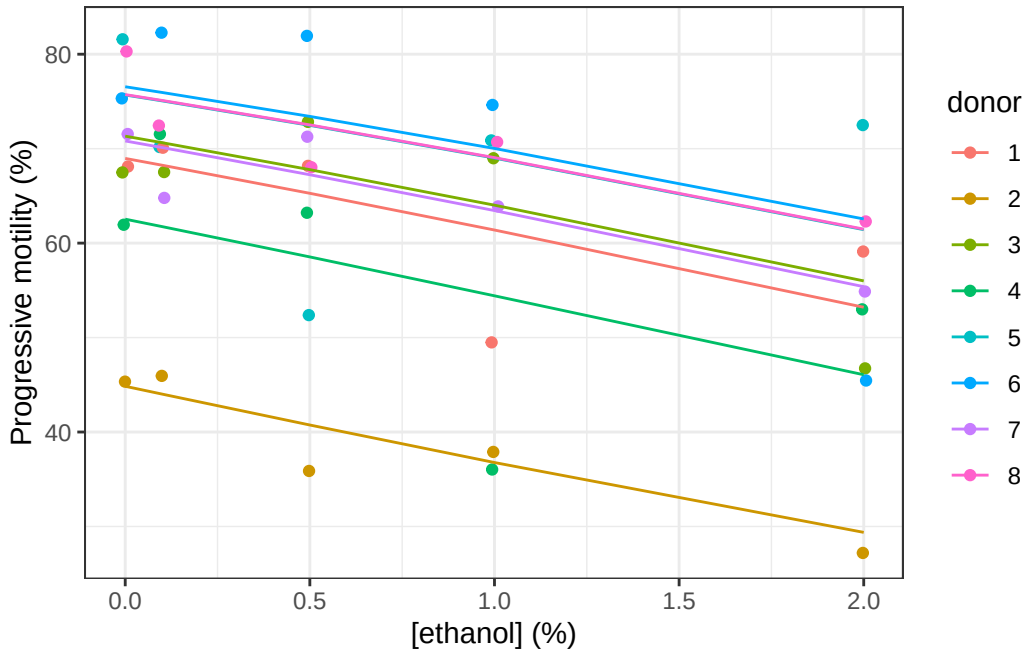
The likelihood ratio test does not detects significant differences between the full and simplified models at $\alpha = 5\%$. We could thus use the simplest `mp_etoh_m2` model with only a shift in the slope per donor. This model is:

,

$$
\begin{aligned}
\text{prog}_i &\sim \text{Binomial}(n = 1, \text{prob}_{\text{prog}=1} = \widehat{P}) \\
, \log\left[\frac{\hat{P}}{1-\hat{P}}\right] &= \alpha_{j[i]} + \beta_1(\text{conc}) \qquad , \qquad (4) \\
\alpha_j &\sim N\left(\mu_{\alpha_j}, \sigma^2_{\alpha_j}\right), \text{ for donor j} = 1, \ldots, \text{J}
\end{aligned}
$$

Here is a plot of this model:

```
ggplot(data = mp_etoh) +
  geom_jitter(aes(x = conc, y = prog_frac * 100, col = donor),
    width = 0.01) +
  geom_line(aes(x = conc, y = fitted(mp_etoh_m2) * 100, col = donor)) +
  labs(x = "[ethanol] (%)", y = "Progressive motility (%)")
```




```
summary(mp_etoh_m2)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: cbind(prog, total - prog) ~ conc + (1 | donor)
```

```
   Data: mp_etoh

     AIC      BIC   logLik deviance df.resid
   341.0    346.1   -167.5    335.0       37

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.3068 -0.7902  0.1791  0.9763  2.4924

Random effects:
 Groups Name        Variance Std.Dev.
 donor  (Intercept) 0.1947   0.4413
Number of obs: 40, groups:  donor, 8

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.79579    0.16194   4.914 8.93e-07 ***
conc        -0.33502    0.04006  -8.362  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
conc -0.182
```

The Z test indicates that `conc` is significantly different from zero at $\alpha = 5\%$. However, it is not the best test in the case of a mixed model like here. We prefer to rely on the 95% confidence interval calculated either on the profile, or via parametric bootstrap (and especially the later one):

```
confint(mp_etoh_m2, level = 0.95) # 95% CI based on profile
```

```
Computing profile confidence intervals ...

                2.5 %     97.5 %
.sig01       0.2816489  0.8030597
(Intercept)  0.4397141  1.1543188
conc        -0.4136293 -0.2565405
```

```r
set.seed(2784)
# 1000x parameter bootstrap
(mp_etoh_m2_conf <- confint(mp_etoh_m2, level = 0.95,
  method = "boot", nsim  = 1000L))
```

```
Computing bootstrap confidence intervals ...
```

```
1 warning(s): Model failed to converge with max|grad| = 0.00694994 (tol = 0.002, component 1)
```

```
                 2.5 %     97.5 %
.sig01       0.1895877  0.6227712
(Intercept)  0.4836082  1.1217895
conc        -0.4130732 -0.2636878
```

We had one model with singularity among the 1000, not a big problem (we may ignore this warning). Slope for `conc` is significantly different from zero at $\alpha = 5\%$ because the 95% CI does not contain zero.

Additional verifications

We could double-check the significance of the slope `conc` by looking at a likelihood ratio test when dropping `conc` from the model:

```r
#drop1(mp_etoh_m2, scope = "conc")
mp_etoh_m3 <- glmer(cbind(prog, total - prog) ~ 1 + (1 | donor),
  data = mp_etoh, family = binomial(link = "logit"))
anova(mp_etoh_m2, mp_etoh_m3, refit = FALSE)
```

```
Data: mp_etoh
Models:
mp_etoh_m3: cbind(prog, total - prog) ~ 1 + (1 | donor)
mp_etoh_m2: cbind(prog, total - prog) ~ conc + (1 | donor)
           npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
mp_etoh_m3    2 409.02 412.40 -202.51   405.02
mp_etoh_m2    3 341.03 346.09 -167.51   335.03 69.995  1  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The model with `conc` is significantly different at α level 5% from a reference model that sets the slope `conc` = 0. There is thus a significant effect of ethanol concentration (confirmation of results obtained from 95% CI).

We also double-check convergence of the model by trying different optimisation engines (just to make sure). First, is there a singularity in the model?

```
isSingular(mp_etoh_m2)
```

```
[1] FALSE
```

… then, a report about the model convergence:

```
mp_etoh_m2_all <- allFit(mp_etoh_m2)
```

```
bobyqa : [OK]
Nelder_Mead : [OK]
nlminbwrap : [OK]
nmkbw : [OK]
optimx.L-BFGS-B : [OK]
nloptwrap.NLOPT_LN_NELDERMEAD : [OK]
nloptwrap.NLOPT_LN_BOBYQA : [OK]
```

```
summary(mp_etoh_m2_all)
```

```
$which.OK
                         bobyqa                     Nelder_Mead
                           TRUE                            TRUE
                     nlminbwrap                          nmkbw
                           TRUE                            TRUE
                optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                           TRUE                            TRUE
    nloptwrap.NLOPT_LN_BOBYQA
                           TRUE

$msgs
$msgs$bobyqa
NULL
```

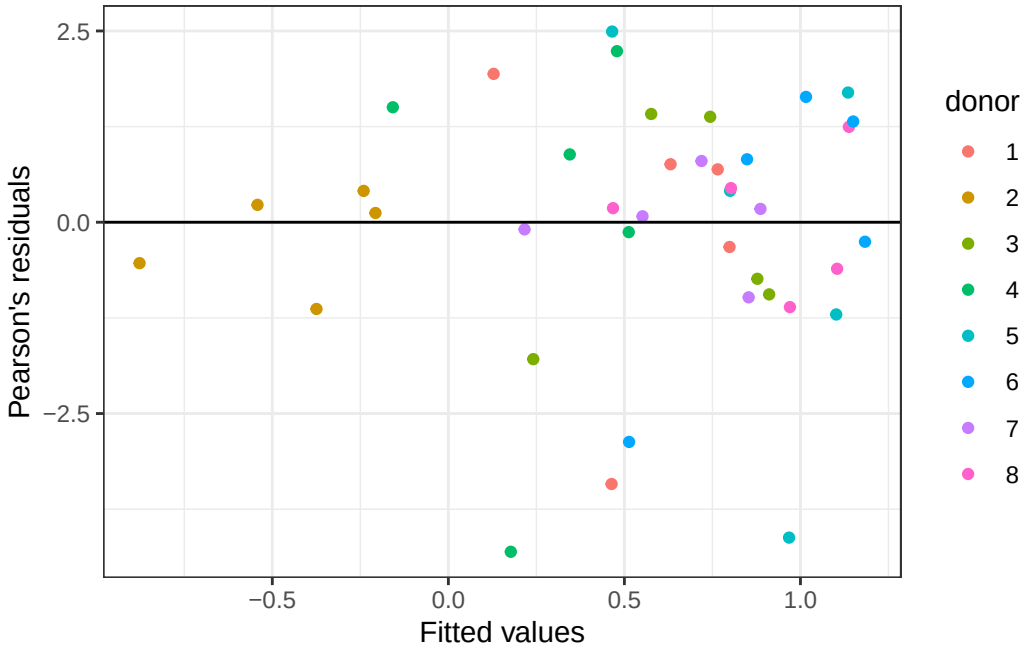
```
$msgs$Nelder_Mead
NULL

$msgs$nlminbwrap
NULL

$msgs$nmkbw
NULL

$msgs$`optimx.L-BFGS-B`
NULL

$msgs$nloptwrap.NLOPT_LN_NELDERMEAD
NULL

$msgs$nloptwrap.NLOPT_LN_BOBYQA
NULL


$fixef
                              (Intercept)       conc
bobyqa                          0.7957873 -0.3350232
Nelder_Mead                     0.7957923 -0.3350248
nlminbwrap                      0.7957905 -0.3350226
nmkbw                           0.7957880 -0.3349973
optimx.L-BFGS-B                 0.7957876 -0.3350233
nloptwrap.NLOPT_LN_NELDERMEAD   0.7957571 -0.3350295
nloptwrap.NLOPT_LN_BOBYQA       0.7957865 -0.3350226

$llik
                       bobyqa                     Nelder_Mead
                    -167.5134                       -167.5134
                   nlminbwrap                           nmkbw
                    -167.5134                       -167.5134
              optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                    -167.5134                       -167.5134
    nloptwrap.NLOPT_LN_BOBYQA
                    -167.5134

$sdcor
                              donor.(Intercept)
bobyqa                                0.4412697
Nelder_Mead                           0.4412728
```

```
nlminbwrap                             0.4412718
nmkbw                                  0.4413067
optimx.L-BFGS-B                        0.4412720
nloptwrap.NLOPT_LN_NELDERMEAD          0.4412887
nloptwrap.NLOPT_LN_BOBYQA              0.4412697

$theta
                              donor.(Intercept)
bobyqa                                0.4412697
Nelder_Mead                           0.4412728
nlminbwrap                            0.4412718
nmkbw                                 0.4413067
optimx.L-BFGS-B                       0.4412720
nloptwrap.NLOPT_LN_NELDERMEAD         0.4412887
nloptwrap.NLOPT_LN_BOBYQA             0.4412697

$times
                              user.self sys.self elapsed user.child sys.child
bobyqa                            0.041    0.000   0.041          0         0
Nelder_Mead                       0.058    0.000   0.059          0         0
nlminbwrap                        0.052    0.000   0.052          0         0
nmkbw                             0.064    0.000   0.064          0         0
optimx.L-BFGS-B                   0.351    0.001   0.353          0         0
nloptwrap.NLOPT_LN_NELDERMEAD     0.055    0.000   0.056          0         0
nloptwrap.NLOPT_LN_BOBYQA         0.039    0.000   0.040          0         0

$feval
                         bobyqa                     Nelder_Mead
                             48                              95
                      nlminbwrap                           nmkbw
                             NA                             104
                optimx.L-BFGS-B nloptwrap.NLOPT_LN_NELDERMEAD
                             14                              94
    nloptwrap.NLOPT_LN_BOBYQA
                             30

attr(,"class")
[1] "summary.allFit"
```

Analysis of the residuals

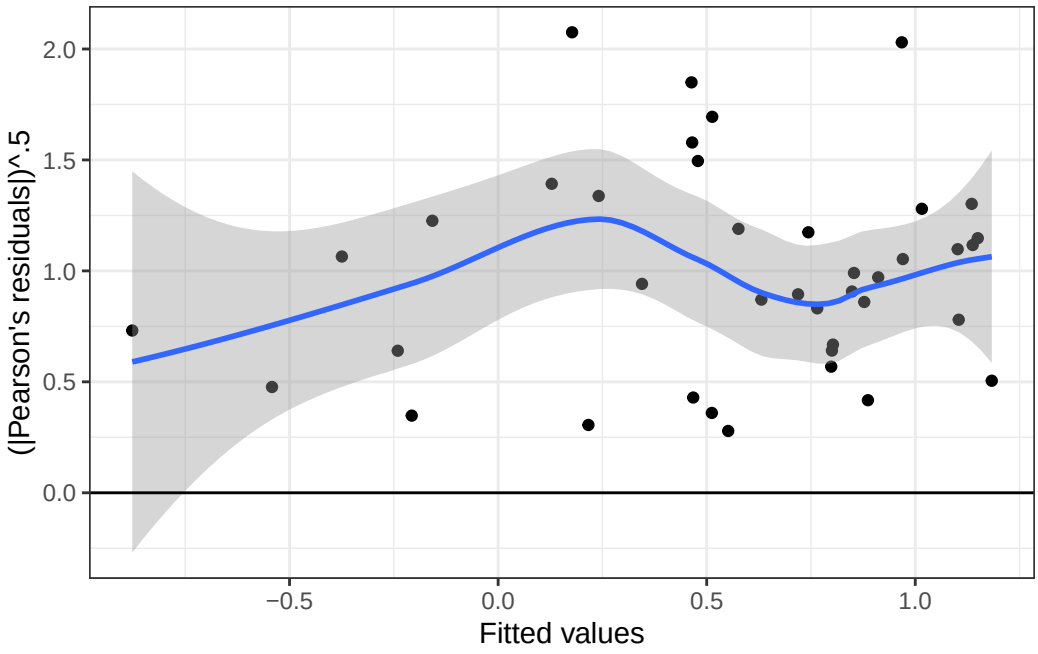
Let's check how the residuals distribute and if there is homoscedasticity.

```
mp_etoh <- fortify.merMod(mp_etoh_m2)
ggplot(data = mp_etoh, aes(x = .fitted, y = .scresid, col = donor)) +
  geom_point() +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "Pearson's residuals")
```



There is one extreme value, but otherwise, residuals seem rather correctly distributed. Linearity is good here.

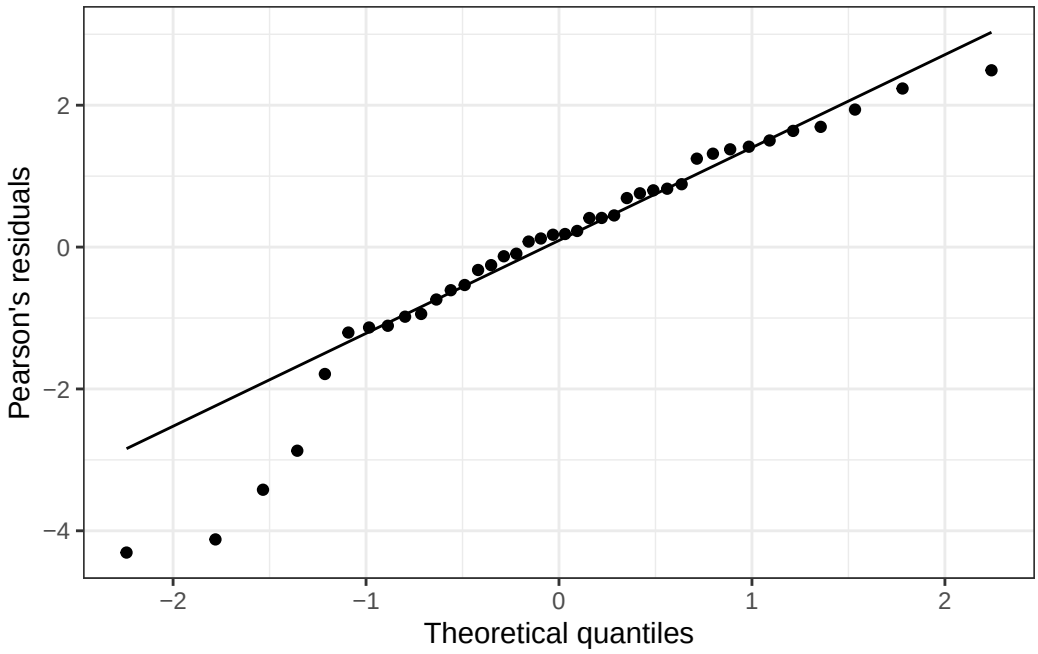
```
ggplot(data = mp_etoh, aes(x = .fitted, y = sqrt(abs(.scresid)))) +
  geom_point() +
  geom_smooth(method = "loess", formula = y ~ x) +
  geom_hline(yintercept = 0) +
  labs(x = "Fitted values", y = "(|Pearson's residuals|)^.5")
```



Homoscedasticity of the residuals seems here acceptable (the blue curve that is a loess smoothing in the data is relatively horizontal).

With the same remark as for DMSO, here is the quantile-quantile plot of the residuals:

```
ggplot(data = mp_etoh, aes(sample = .scresid)) +
  geom_qq() +
  geom_qq_line() +
  labs(x = "Theoretical quantiles", y = "Pearson's residuals")
```



It appears not Normal for. A Shapiro-Wilk test confirms non Normality:

```
shapiro.test(mp_etoh$.scresid)
```

```
    Shapiro-Wilk normality test

data:  mp_etoh$.scresid
W = 0.92514, p-value = 0.01121
```

Predictions

The model allows to calculate the drop in progressive mobility according to ethanol concentration from 0 to 2%. Note that an inverse logit transformation is required. Here is an example:

```
mp_etoh_slope <- c(
  ci95_min   = min(mp_etoh_m2_conf["conc", ]),
  estimate = fixef(mp_etoh_m2)[["conc"]],
  ci95_max  = max(mp_etoh_m2_conf["conc", ]))
mp_etoh_slope
```

```
  ci95_min   estimate   ci95_max
-0.4130732 -0.3350244 -0.2636878
```

```
#saveRDS(mp_etoh_slope, "../data/motility_prog_ETOH_slope.rds")
```

Let's say we want to calculate the drop in progressive mobility for various ethanol concentrations between 0 and 2% if the progressive mobility in a sample without ethanol is 80%. The calculation is:

```
predict_logit <- function(conc, intercept = 1, slopes) {
  slopes_mat <- matrix(slopes, nrow = 1,
    dimnames = list(NULL, names(slopes)))
  data.frame(conc = conc, -intercept +
    boot::inv.logit(boot::logit(intercept) +
    conc %*% slopes_mat))
}
etoh_conc <- (0:20) / 10
mp_etoh_lost <- predict_logit(etoh_conc, 0.8, mp_etoh_slope)
mp_etoh_lost
```

```
   conc     ci95_min     estimate     ci95_max
1   0.0  0.000000000  0.000000000  0.000000000
2   0.1 -0.006691138 -0.005414301 -0.004252397
3   0.2 -0.013546378 -0.010936529 -0.008571636
4   0.3 -0.020565768 -0.016566750 -0.012957769
5   0.4 -0.027749071 -0.022304912 -0.017410801
6   0.5 -0.035095756 -0.028150837 -0.021930692
7   0.6 -0.042604977 -0.034104214 -0.026517350
8   0.7 -0.050275572 -0.040164602 -0.031170638
9   0.8 -0.058106045 -0.046331419 -0.035890365
10  0.9 -0.066094558 -0.052603943 -0.040676287
11  1.0 -0.074238925 -0.058981303 -0.045528109
12  1.1 -0.082536602 -0.065462483 -0.050445480
13  1.2 -0.090984682 -0.072046313 -0.055427992
14  1.3 -0.099579891 -0.078731468 -0.060475183
15  1.4 -0.108318583 -0.085516469 -0.065586531
16  1.5 -0.117196743 -0.092399677 -0.070761457
17  1.6 -0.126209984 -0.099379293 -0.075999323
18  1.7 -0.135353550 -0.106453359 -0.081299428
19  1.8 -0.144622323 -0.113619756 -0.086661014
20  1.9 -0.154010826 -0.120876203 -0.092083261
```

```
21  2.0 -0.163513232 -0.128220261 -0.097565288
```

```
#saveRDS(mp_etoh_lost, "../data/motility_prog_ETOH_lost.rds")
```

This is the lost in progressive mobility that the model predicts.

General informations

```
sessionInfo()
```

```
R version 4.1.3 (2022-03-10)
Platform: x86_64-apple-darwin17.0 (64-bit)
Running under: macOS Big Sur/Monterey 10.16

Matrix products: default
LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] ggplot2_3.3.5 lme4_1.1-29   Matrix_1.4-1

loaded via a namespace (and not attached):
 [1] tidyr_1.2.0         jsonlite_1.8.0      splines_4.1.3
 [4] equatiomatic_0.3.1  shiny_1.7.1         assertthat_0.2.1
 [7] highr_0.9           broom.mixed_0.2.9.4 cellranger_1.1.0
[10] yaml_2.3.5          globals_0.14.0      numDeriv_2016.8-1.1
[13] pillar_1.7.0        backports_1.4.1     lattice_0.20-45
[16] glue_1.6.2          digest_0.6.29       promises_1.2.0.1
[19] minqa_1.2.4         colorspace_2.0-3    dfoptim_2020.10-1
[22] htmltools_0.5.2     httpuv_1.6.5        pkgconfig_2.0.3
[25] broom_0.8.0         listenv_0.8.0       purrr_0.3.4
[28] xtable_1.8-4        scales_1.2.0        later_1.3.0
[31] tibble_3.1.6        mgcv_1.8-40         generics_0.1.2
[34] farver_2.1.0        ellipsis_0.3.2      withr_2.5.0
[37] furrr_0.2.3         repr_1.1.4          skimr_2.1.4
```

```
[40] cli_3.2.0           magrittr_2.0.3      crayon_1.5.1
[43] readxl_1.4.0        mime_0.12           evaluate_0.15
[46] fansi_1.0.3         future_1.24.0       parallelly_1.31.0
[49] nlme_3.1-157        MASS_7.3-56         forcats_0.5.1
[52] tools_4.1.3         lifecycle_1.0.1     stringr_1.4.0
[55] munsell_0.5.0       compiler_4.1.3      rlang_1.0.2
[58] grid_4.1.3          nloptr_2.0.0        rstudioapi_0.13
[61] base64enc_0.1-3     labeling_0.4.2      rmarkdown_2.13
[64] boot_1.3-28         gtable_0.3.0        codetools_0.2-18
[67] DBI_1.1.2           R6_2.5.1            knitr_1.38
[70] dplyr_1.0.8         optimx_2021-10.12   fastmap_1.1.0
[73] utf8_1.2.2          stringi_1.7.6       parallel_4.1.3
[76] Rcpp_1.0.8.3        vctrs_0.4.1         tidyselect_1.1.2
[79] xfun_0.30
```