Host protein and MAA concentration-Mixed effects model

Code ▾

Or Ben-Zvi

load packages

Hide

```
library(car)
library(ggplot2)
library(lme4)
library(emmeans)
library(MASS)
library(cowplot)
library(dplyr)
library(doBy)
```

read data

Hide

```
data_hpmaa <- read.csv(file.choose())
```

Hide

```
str(data_hpmaa)
```

```
'data.frame':   79 obs. of  13 variables:
 $ id       : Factor w/ 79 levels "GA1","GA2","GA3",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ treatment: Factor w/ 2 levels "par","paruv": 1 1 1 2 2 2 1 1 1 2 ...
 $ morph    : Factor w/ 3 levels "green","red",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ colony   : Factor w/ 14 levels "a","b","c","d",..: 1 1 1 1 1 1 2 2 2 2 ...
 $ tank     : int  1 2 3 4 5 6 1 2 3 4 ...
 $ maat0    : Factor w/ 76 levels "#DIV/0!","10.62034265",..: 6 11 10 22 12 14 23 36 65 26 ...
 $ maat1    : Factor w/ 77 levels "1.049626333",..: 17 15 13 44 40 64 22 11 51 59 ...
 $ dmaa     : Factor w/ 74 levels "-1.15E+00","-1.21E+00",..: 15 46 49 74 56 64 50 28 20 55 ...
 $ pmaa     : Factor w/ 76 levels "-10.32869565",..: 48 8 13 59 67 74 7 42 17 62 ...
 $ hpt0     : Factor w/ 77 levels "10.25148515",..: 18 44 28 66 57 49 67 69 41 65 ...
 $ hpt1     : Factor w/ 73 levels "1.308888889",..: 62 71 41 13 70 22 69 33 7 17 ...
 $ dhp      : Factor w/ 72 levels "-0.03130911",..: 62 10 7 30 25 11 34 36 52 29 ...
 $ php      : Factor w/ 72 levels "-0.287346704",..: 68 30 2 45 42 26 47 46 24 41 ...
```

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```
data_hpmaa$hpt0 <- as.numeric(as.character(data_hpmaa$hpt0))
data_hpmaa$hpt1 <- as.numeric(as.character(data_hpmaa$hpt1))
data_hpmaa$maat0 <- as.numeric(as.character(data_hpmaa$maat0))
data_hpmaa$maat1 <- as.numeric(as.character(data_hpmaa$maat1))
data_hpmaa$dhp <- as.numeric(as.character(data_hpmaa$dhp))
data_hpmaa$dmaa <- as.numeric(as.character(data_hpmaa$dmaa))
data_hpmaa$php <- as.numeric(as.character(data_hpmaa$php))
data_hpmaa$pmaa <- as.numeric(as.character(data_hpmaa$pmaa))
data_hpmaa$tank <- as.factor(data_hpmaa$tank)
str(data_hpmaa)
```

```
'data.frame':   79 obs. of  13 variables:
 $ id       : Factor w/ 79 levels "GA1","GA2","GA3",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ treatment: Factor w/ 2 levels "par","paruv": 1 1 1 2 2 2 1 1 1 2 ...
 $ morph    : Factor w/ 3 levels "green","red",..: 1 1 1 1 1 1 1 1 1 1 ...
 $ colony   : Factor w/ 14 levels "a","b","c","d",..: 1 1 1 1 1 1 1 2 2 2 2 ...
 $ tank     : Factor w/ 6 levels "1","2","3","4",..: 1 2 3 4 5 6 1 2 3 4 ...
 $ maat0    : num  2.59 3.03 2.95 3.71 3.04 ...
 $ maat1    : num  2.39 2.29 2.09 4.45 4.17 ...
 $ dmaa     : num  -0.205 -0.744 -0.856 0.743 1.13 2.51 -0.91 -3.1 -2.45 1.12 ...
 $ pmaa     : num  -7.88 -24.51 -29.06 20.06 37.01 ...
 $ hpt0     : num  15.6 21.5 17.4 39.6 30.2 ...
 $ hpt1     : num  26.08 9.93 15.47 11.69 9.87 ...
 $ dhp      : num  10.51 -11.55 -1.89 -27.88 -20.37 ...
 $ php      : num  67.6 -53.8 -10.9 -70.5 -67.4 ...
```
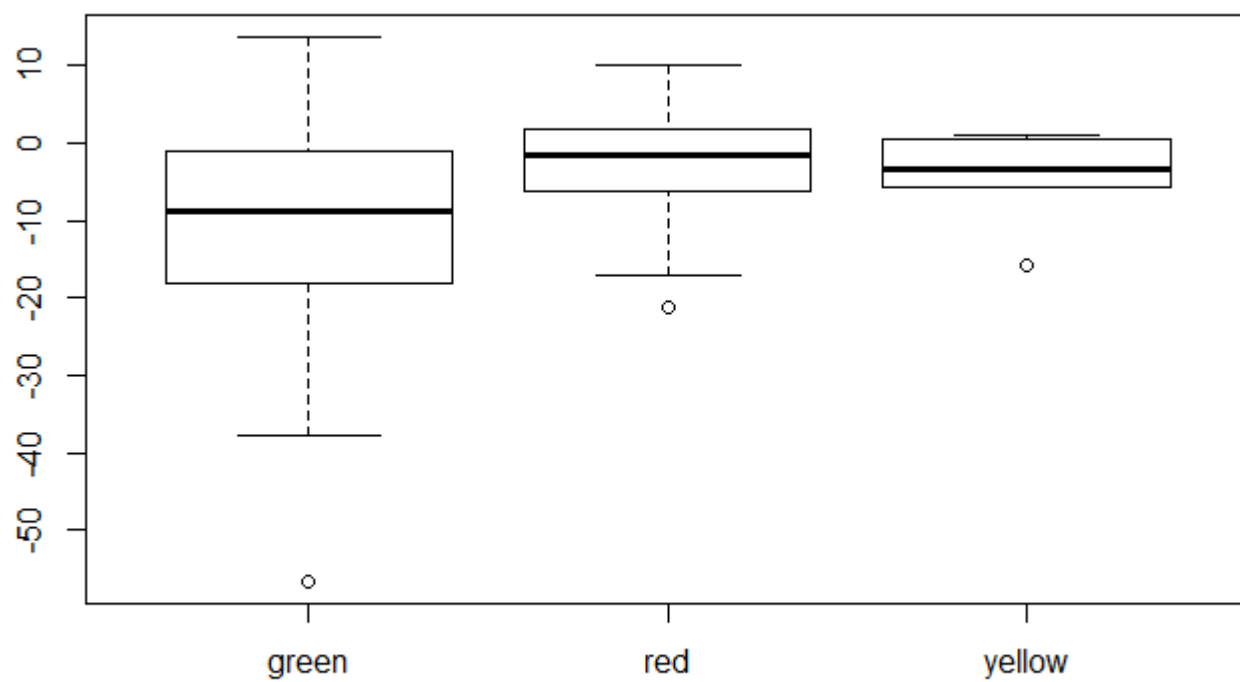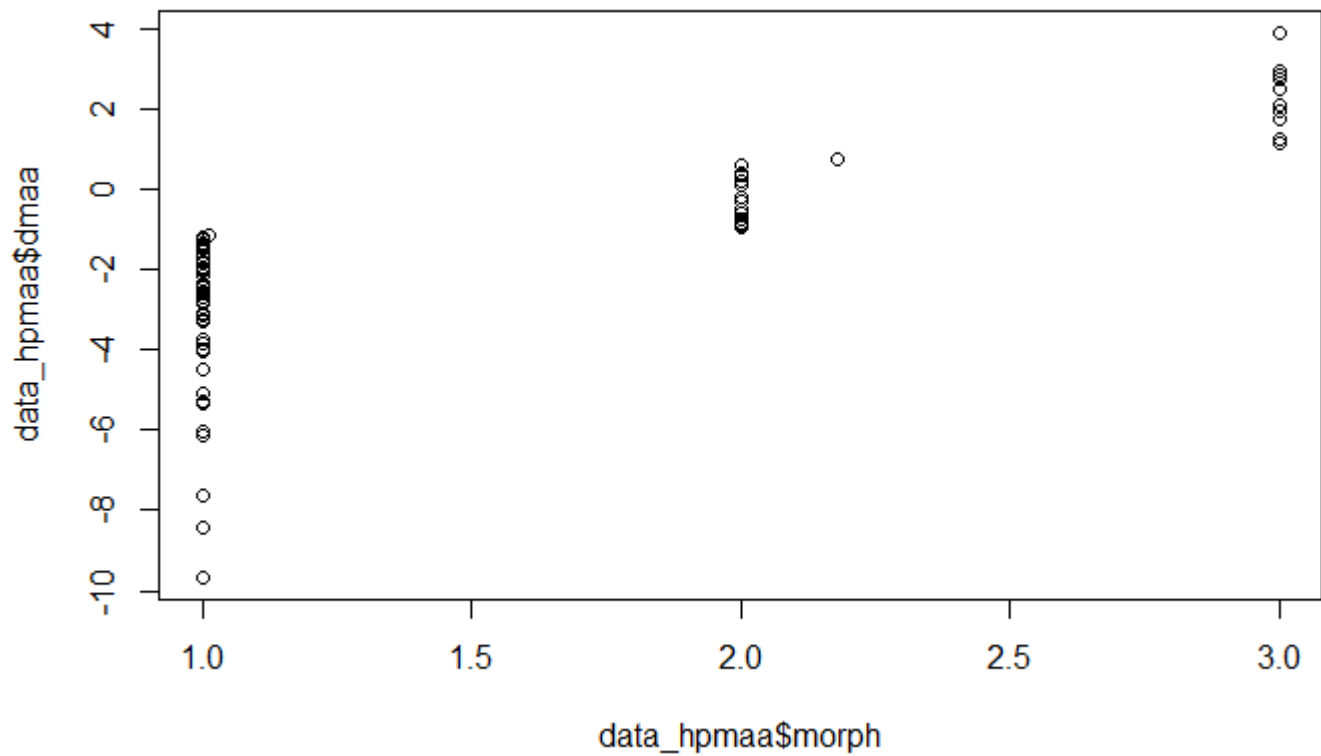
Visualize data

Hide

```
qqplot(data_hpmaa$morph,data_hpmaa$dhp)
```
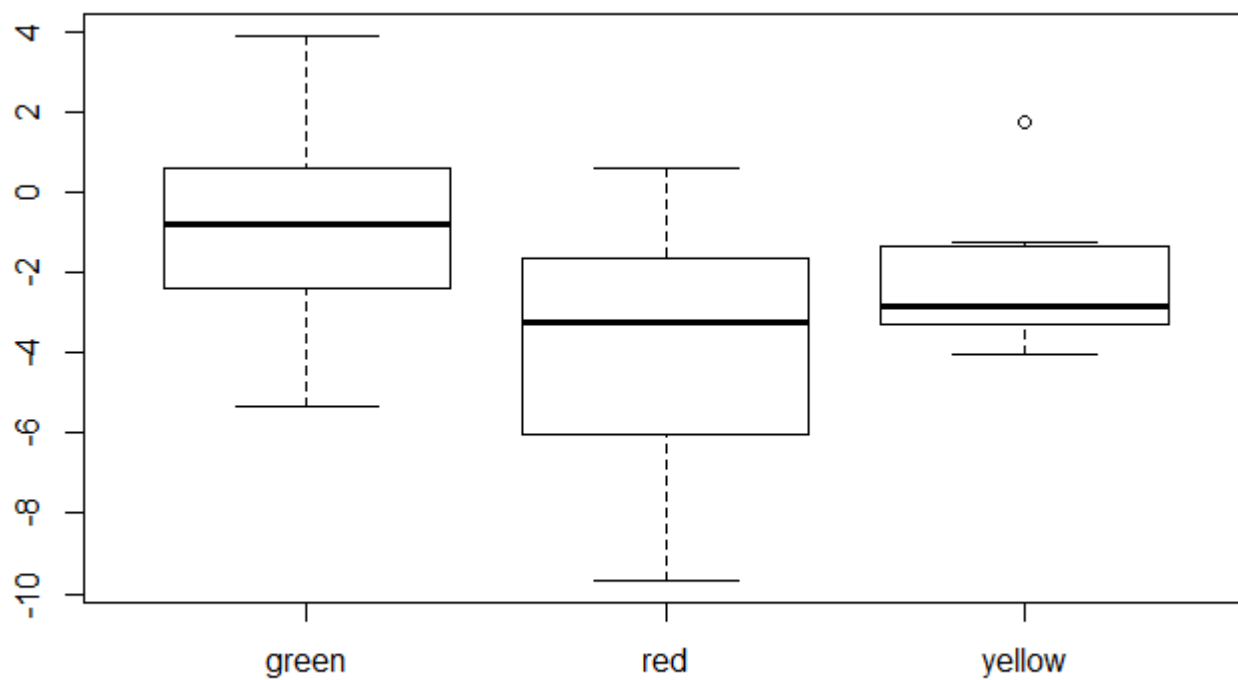
```
plot(data_hpmaa$morph,data_hpmaa$dhp)
```
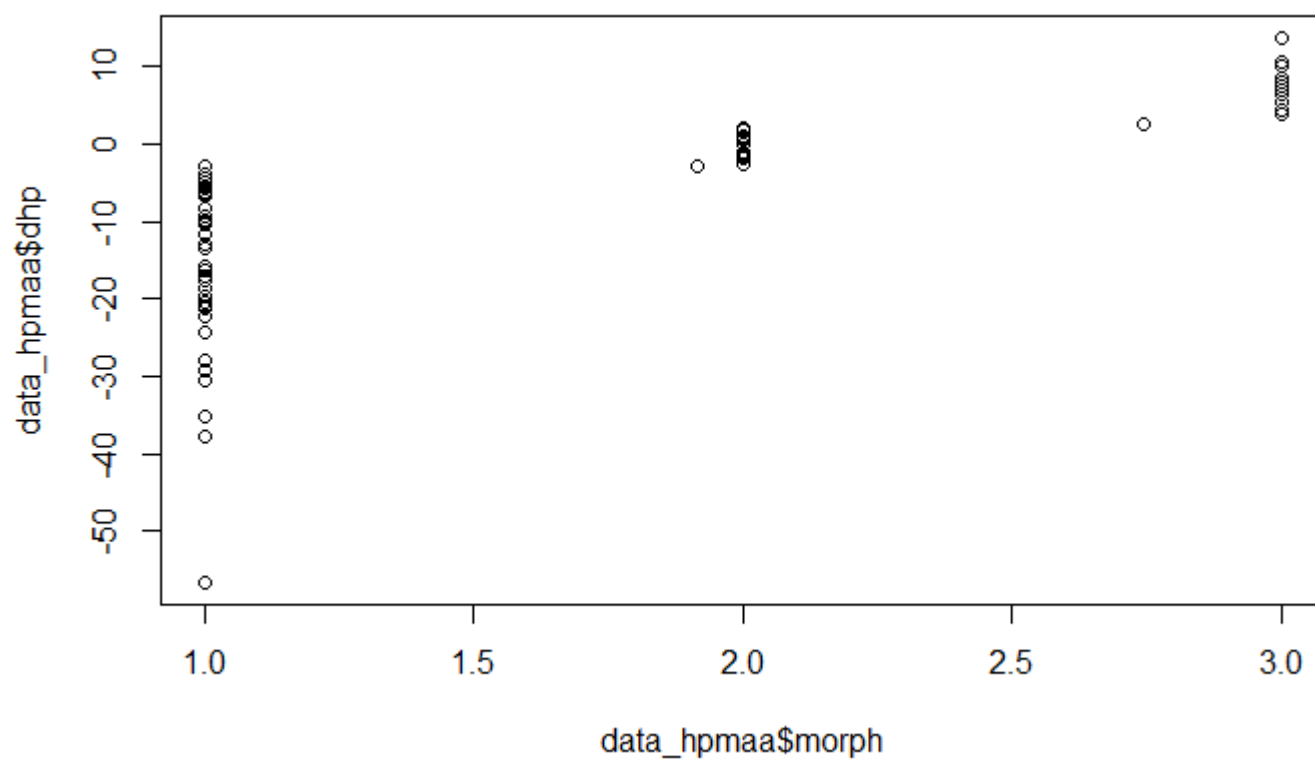
```
qqplot(data_hpmaa$morph,data_hpmaa$dmaa)
```


Hide

```
plot(data_hpmaa$morph,data_hpmaa$dmaa)
```

Test with mixed effect model

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```
model_hp <- lmer(dhp ~ treatment*morph + (1|morph/colony) + (1|treatment/tank) ,
                 data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

Hide

```
summary(model_hp)
```

```
Linear mixed model fit by maximum likelihood  ['lmerMod']
Formula: dhp ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
   Data: data_hpmaa

     AIC       BIC    logLik deviance df.resid
   572.9     597.8    -275.5    550.9       60

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.6089 -0.5205  0.0674  0.5473  1.8754

Random effects:
 Groups          Name        Variance Std.Dev.
 colony:morph    (Intercept)  22.34    4.727
 tank:treatment  (Intercept)   0.00    0.000
 morph           (Intercept)   0.00    0.000
 treatment       (Intercept)   0.00    0.000
 Residual                    120.82   10.992
Number of obs: 71, groups:  colony:morph, 14; tank:treatment, 6; morph, 3; treatment, 2

Fixed effects:
                           Estimate Std. Error t value
(Intercept)                 -9.8052     2.7977  -3.505
treatmentparuv              -0.2481     3.1789  -0.078
morphred                     3.1616     5.3572   0.590
morphyellow                  4.9310     6.3187   0.780
treatmentparuv:morphred      6.9688     6.2249   1.119
treatmentparuv:morphyellow   0.4473    12.8195   0.035

Correlation of Fixed Effects:
              (Intr) trtmnt mrphrd mrphyl trtmntprv:mrphr
treatmntprv   -0.566
morphred      -0.522  0.296
morphyellow   -0.443  0.251  0.231
trtmntprv:mrphr  0.289 -0.511 -0.554 -0.128
trtmntprv:mrphy  0.140 -0.248 -0.073 -0.351  0.127
convergence code: 0
singular fit
```

Hide

```
model_hp1 <- lmer(dhp ~ treatment + (1|morph/colony) + (1|treatment/tank) ,
                  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

Hide

```
model_hp2 <- lmer(dhp ~ morph + (1|morph/colony) + (1|treatment/tank) ,
                  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

Hide

```
anova(model_hp, model_hp1)
```

```
Data: data_hpmaa
Models:
model_hp1: dhp ~ treatment + (1 | morph/colony) + (1 | treatment/tank)
model_hp: dhp ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
          Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model_hp1  7 568.51 584.35 -277.25   554.51
model_hp  11 572.92 597.81 -275.46   550.92 3.5868      4     0.4648
```

Hide

```
anova(model_hp, model_hp2)
```

```
Data: data_hpmaa
Models:
model_hp2: dhp ~ morph + (1 | morph/colony) + (1 | treatment/tank)
model_hp: dhp ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
          Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model_hp2  8 568.48 586.59 -276.24   552.48
model_hp  11 572.92 597.81 -275.46   550.92 1.5628      3     0.6678
```

Hide

```
model_maa <- lmer(dmaa ~ treatment*morph + (1|morph/colony) + (1|treatment/tank) ,
                  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

Hide

```
summary(model_maa)
```

```
Linear mixed model fit by maximum likelihood  ['lmerMod']
Formula: dmaa ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
   Data: data_hpmaa

    AIC      BIC   logLik deviance df.resid
  341.9    367.2   -159.9    319.9       63

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.6095 -0.5767  0.1880  0.6077  2.0744

Random effects:
 Groups          Name        Variance Std.Dev.
 colony:morph    (Intercept) 0.4243   0.6514
 tank:treatment  (Intercept) 0.1041   0.3226
 morph           (Intercept) 0.0000   0.0000
 treatment       (Intercept) 0.0000   0.0000
 Residual                    3.9744   1.9936
Number of obs: 74, groups:  colony:morph, 14; tank:treatment, 6; morph, 3; treatment, 2

Fixed effects:
                           Estimate Std. Error t value
(Intercept)                -1.74283    0.50332  -3.463
treatmentparuv              1.96226    0.63364   3.097
morphred                   -1.78639    0.89535  -1.995
morphyellow                -0.04146    1.02326  -0.041
treatmentparuv:morphred    -2.53934    1.12886  -2.249
treatmentparuv:morphyellow -3.37762    1.53059  -2.207

Correlation of Fixed Effects:
            (Intr) trtmnt mrphrd mrphyl trtmntprv:mrphr
treatmntprv    -0.628
morphred       -0.485  0.292
morphyellow    -0.425  0.255  0.239
trtmntprv:mrphr  0.291 -0.464 -0.601 -0.143
trtmntprv:mrphy  0.215 -0.342 -0.121 -0.519  0.192
convergence code: 0
singular fit
```

Hide

```
model_maa1 <- lmer(dmaa ~ treatment + (1|morph/colony) + (1|treatment/tank) ,
                  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

Hide

```
model_maa2 <- lmer(dmaa ~ morph + (1|morph/colony) + (1|treatment/tank) ,
                  data=data_hpmaa, REML=FALSE)
```

```
singular fit
```

```
anova(model_maa, model_maa1)
```

```
Data: data_hpmaa
Models:
model_maa1: dmaa ~ treatment + (1 | morph/colony) + (1 | treatment/tank)
model_maa: dmaa ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
            Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model_maa1   7 349.05 365.17 -167.52   335.05
model_maa   11 341.87 367.21 -159.93   319.87 15.177      4   0.004348 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(model_maa, model_maa2)
```

```
Data: data_hpmaa
Models:
model_maa2: dmaa ~ morph + (1 | morph/colony) + (1 | treatment/tank)
model_maa: dmaa ~ treatment * morph + (1 | morph/colony) + (1 | treatment/tank)
            Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model_maa2   8 346.25 364.69 -165.13   330.25
model_maa   11 341.87 367.21 -159.93   319.87 10.384      3    0.01557 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
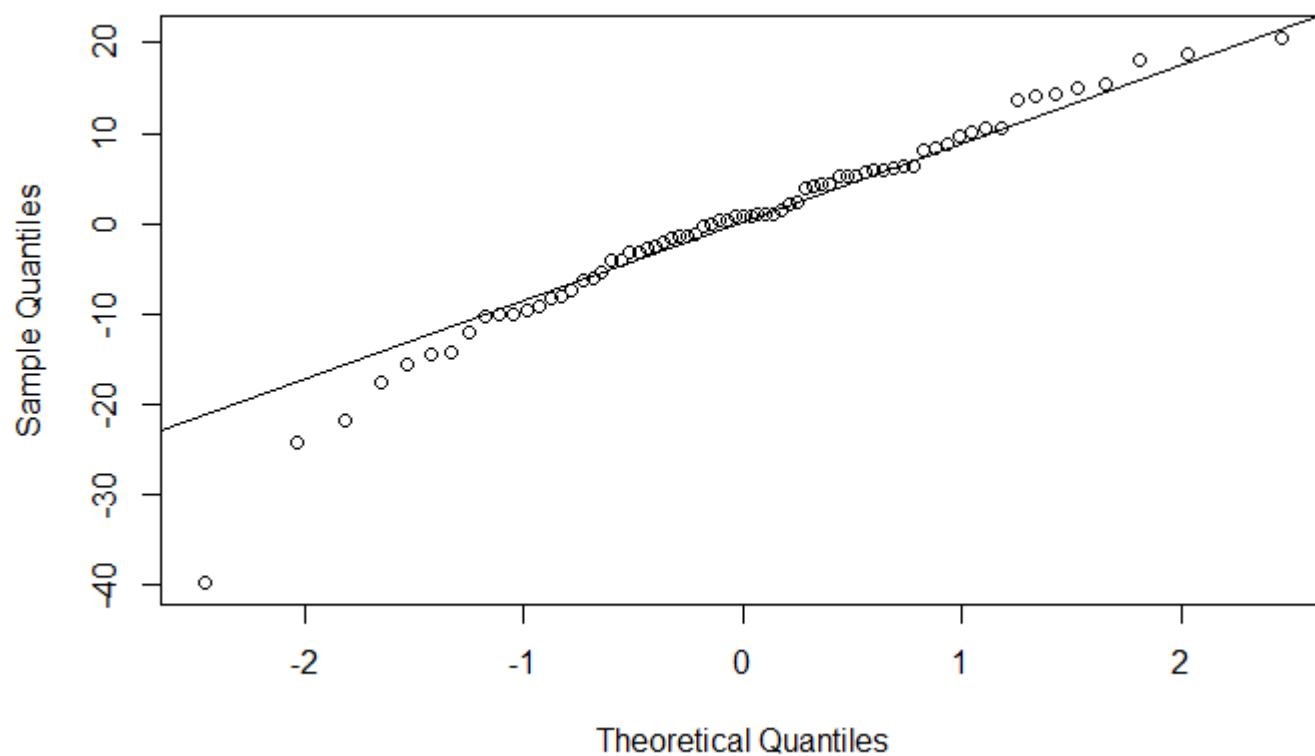
Check the LMM assumptions

Check the normality of model residuals

```
qqnorm(resid(model_hp))
qqline(resid(model_hp))
```

Normal Q-Q Plot


```
qqnorm(resid(model_maa))
qqline(resid(model_maa))
```

Normal Q-Q Plot
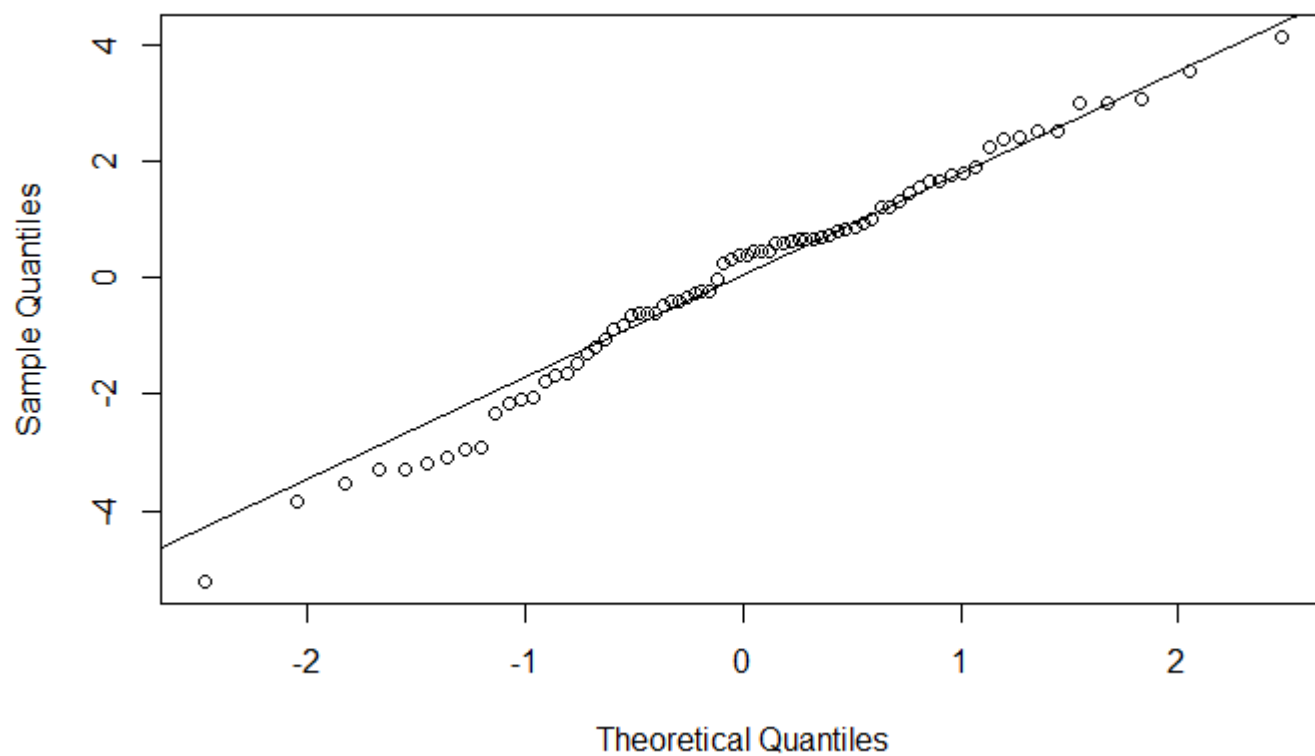
Check for homogenity of variance

Hide

```
plot(model_hp)
```
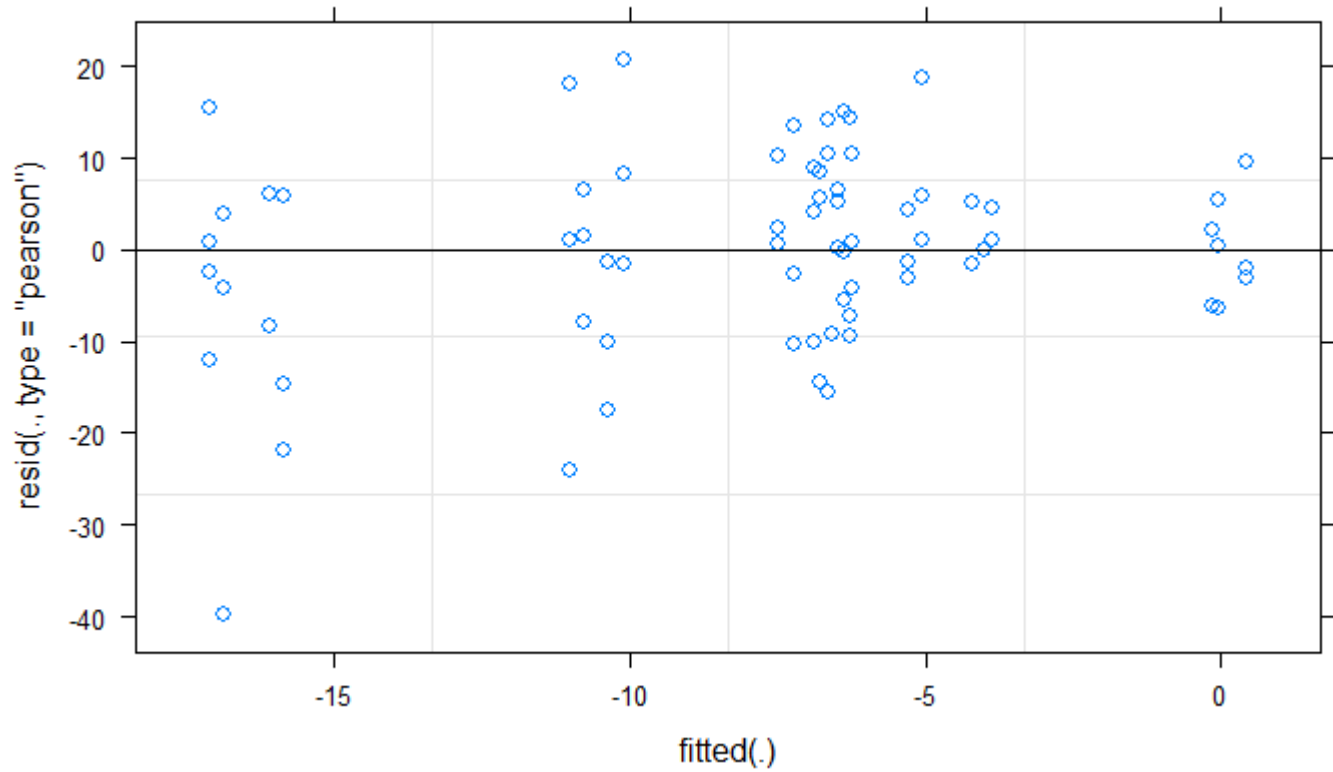

Hide

```
plot(model_maa)
```
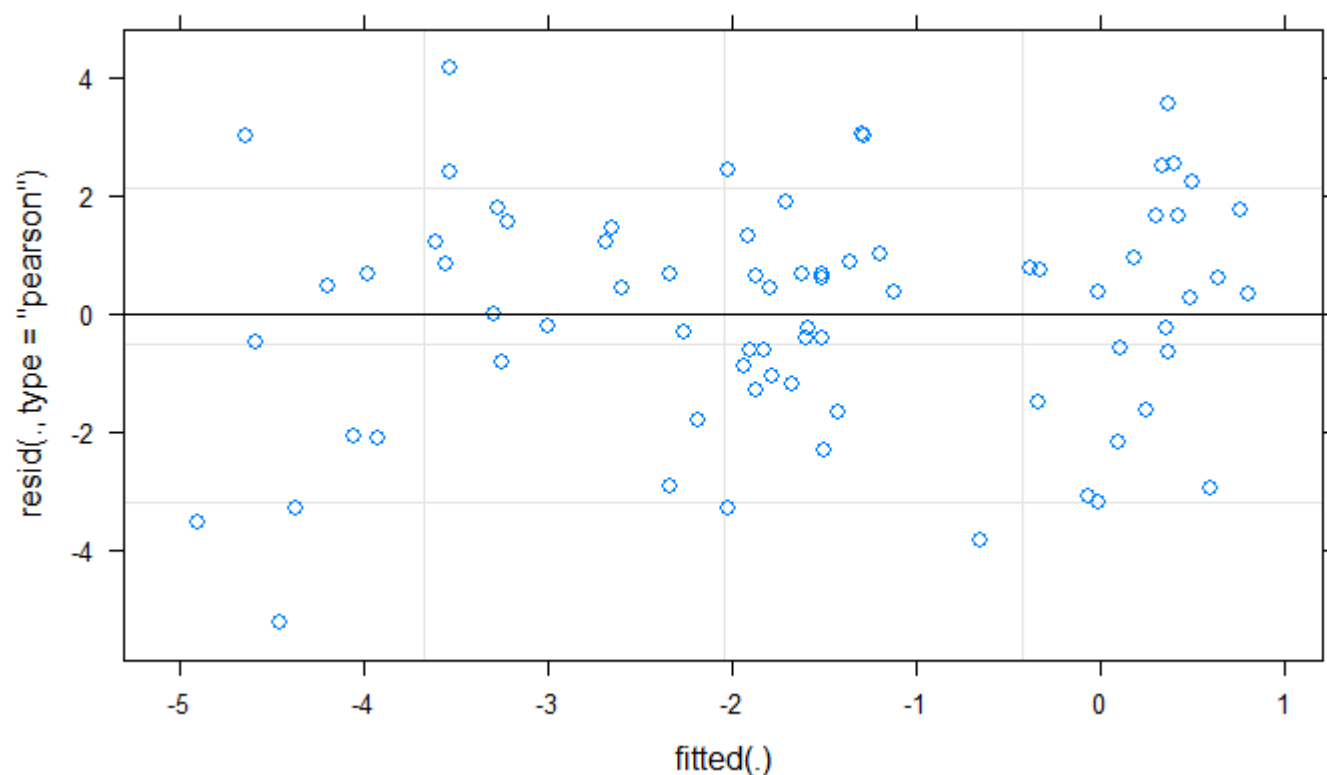
Obtain mean and SD

```
sumfun <- function(x, ...){
  c(mean=mean(x, na.rm=TRUE, ...), sd=sd(x, na.rm=TRUE, ...), l=length(x))
}
mean(data_hpmaa$hpt0, na.rm = TRUE)
```

```
[1] 21.67733
```

```
sd(data_hpmaa$hpt0, na.rm = TRUE)
```

```
[1] 11.15114
```

```
mean(data_hpmaa$hpt1, na.rm = TRUE)
```

```
[1] 13.80183
```

```
sd(data_hpmaa$hpt1, na.rm = TRUE)
```

```
[1] 4.568941
```

Hide

```
summaryBy(hpt0 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	treatment <fctr>	hpt0.mean <dbl>	hpt0.sd <dbl>	hpt0.l <dbl>
1	green	par	23.79227	13.165150	24
2	green	paruv	24.08591	11.808555	24
3	red	par	21.83404	9.060078	9
4	red	paruv	13.16696	3.442265	9
5	yellow	par	18.12794	8.097353	7
6	yellow	paruv	17.41583	2.038584	6

6 rows

Hide

```
summaryBy(hpt0 ~morph, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	hpt0.mean <dbl>	hpt0.sd <dbl>	hpt0.l <dbl>
1	green	23.93909	12.372401	48
2	red	17.75541	8.130852	18
3	yellow	17.86899	6.380917	13

3 rows

Hide

```
summaryBy(hpt0 ~treatment, data=data_hpmaa, FUN=sumfun)
```

	treatment <fctr>	hpt0.mean <dbl>	hpt0.sd <dbl>	hpt0.l <dbl>
1	par	22.36041	11.56206	40
2	paruv	20.91836	10.78772	39

2 rows

Hide

```
summaryBy(hpt1 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	treatment <fctr>	hpt1.mean <dbl>	hpt1.sd <dbl>	hpt1.l <dbl>
1	green	par	13.98704	5.885944	24
2	green	paruv	13.98997	3.510027	24
3	red	par	15.19037	4.967352	9
4	red	paruv	13.34032	3.781468	9
5	yellow	par	10.70346	2.117577	7
6	yellow	paruv	11.98981	NA	6

6 rows

Hide

```
summaryBy(hpt1 ~morph, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	hpt1.mean <dbl>	hpt1.sd <dbl>	hpt1.l <dbl>
1	green	13.98850	4.794027	48
2	red	14.26534	4.387113	18
3	yellow	10.91785	1.965475	13

3 rows

Hide

```
summaryBy(hpt1 ~treatment, data=data_hpmaa, FUN=sumfun)
```

	treatment <fctr>	hpt1.mean <dbl>	hpt1.sd <dbl>	hpt1.l <dbl>
1	par	13.83999	5.398380	40
2	paruv	13.75917	3.497838	39

2 rows

Hide

```
summaryBy(php ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	treatment <fctr>	php.mean <dbl>	php.sd <dbl>	php.l <dbl>

	morph <fctr>	treatment <fctr>	php.mean <dbl>	php.sd <dbl>	php.l <dbl>
1	green	par	-22.637765	58.92197	24
2	green	paruv	-25.527091	44.47746	24
3	red	par	-18.228766	43.30632	9
4	red	paruv	7.760849	45.01695	9
5	yellow	par	-21.855105	28.62351	7
6	yellow	paruv	-25.002275	*NA*	6

6 rows

Hide

```
summaryBy(php ~morph, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	php.mean <dbl>	php.sd <dbl>	php.l <dbl>
1	green	-24.082428	51.66404	48
2	red	-5.998359	44.75625	18
3	yellow	-22.379633	25.63386	13

3 rows

Hide

```
summaryBy(php ~treatment, data=data_hpmaa, FUN=sumfun)
```

	treatment <fctr>	php.mean <dbl>	php.sd <dbl>	php.l <dbl>
1	par	-21.49055	51.53327	40
2	paruv	-17.44138	45.54969	39

2 rows

Hide

```
summaryBy(maat0 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	treatment <fctr>	maat0.mean <dbl>	maat0.sd <dbl>	maat0.l <dbl>
1	green	par	5.430055	1.632603	24
2	green	paruv	5.054276	1.971125	24

morph	treatment	maat0.mean	maat0.sd	maat0.l
<fctr>	<fctr>	<dbl>	<dbl>	<dbl>
3 red	par	5.846749	2.595752	9
4 red	paruv	6.894724	3.607765	9
5 yellow	par	7.499501	1.410929	7
6 yellow	paruv	6.240481	2.451593	6

6 rows

Hide

```
summaryBy(maat0 ~morph, data=data_hpmaa, FUN=sumfun)
```

morph	maat0.mean	maat0.sd	maat0.l
<fctr>	<dbl>	<dbl>	<dbl>
1 green	5.242166	1.800480	48
2 red	6.339914	3.058458	18
3 yellow	7.041675	1.844183	13

3 rows

Hide

```
summaryBy(maat0 ~treatment, data=data_hpmaa, FUN=sumfun)
```

treatment	maat0.mean	maat0.sd	maat0.l
<fctr>	<dbl>	<dbl>	<dbl>
1 par	5.885964	1.963635	40
2 paruv	5.595065	2.511137	39

2 rows

Hide

```
summaryBy(maat1 ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

morph	treatment	maat1.mean	maat1.sd	maat1.l
<fctr>	<fctr>	<dbl>	<dbl>	<dbl>
1 green	par	3.687744	1.366164	24
2 green	paruv	5.270069	1.528755	24
3 red	par	2.317337	1.263014	9
4 red	paruv	2.915283	1.222423	9

morph <fctr>	treatment <fctr>	maat1.mean <dbl>	maat1.sd <dbl>	maat1.l <dbl>
5 yellow	par	5.846019	1.413096	7
6 yellow	paruv	5.785419	4.032577	6

6 rows

Hide

```
summaryBy(maat1 ~morph, data=data_hpmaa, FUN=sumfun)
```

morph <fctr>	maat1.mean <dbl>	maat1.sd <dbl>	maat1.l <dbl>
1 green	4.478906	1.642037	48
2 red	2.616310	1.244401	18
3 yellow	5.821779	2.555562	13

3 rows

Hide

```
summaryBy(maat1 ~treatment, data=data_hpmaa, FUN=sumfun)
```

treatment <fctr>	maat1.mean <dbl>	maat1.sd <dbl>	maat1.l <dbl>
1 par	3.703538	1.705366	40
2 paruv	4.752996	2.078780	39

2 rows

Hide

```
summaryBy(pmaa ~morph * treatment, data=data_hpmaa, FUN=sumfun)
```

morph <fctr>	treatment <fctr>	pmaa.mean <dbl>	pmaa.sd <dbl>	pmaa.l <dbl>
1 green	par	-28.75316	26.64023	24
2 green	paruv	17.69605	52.44962	24
3 red	par	-53.98800	33.83796	9
4 red	paruv	-53.09773	19.08400	9
5 yellow	par	-20.78543	27.50400	7
6 yellow	paruv	-48.02251	14.85842	6

6 rows

Hide

```
summaryBy(pmaa ~morph, data=data_hpmaa, FUN=sumfun)
```

	morph <fctr>	pmaa.mean <dbl>	pmaa.sd <dbl>	pmaa.l <dbl>
1	green	-5.528554	47.37484	48
2	red	-53.569050	27.05644	18
3	yellow	-29.864453	26.71051	13

3 rows

Hide

```
summaryBy(pmaa ~treatment, data=data_hpmaa, FUN=sumfun)
```

	treatment <fctr>	pmaa.mean <dbl>	pmaa.sd <dbl>	pmaa.l <dbl>
1	par	-33.350781	30.15454	40
2	paruv	-4.118402	54.94876	39

2 rows

Visualize the data as a box plot

Hide

```
hp_plot <- ggplot(data = data_hpmaa, aes(x=morph, y=php)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(title= "Host protein concentration (ugproteinmgtissu) concentration", x="Fluorescence mor
ph", y="Relative change (%)") +
  geom_hline(yintercept=0, color= "red", size = 1.25, alpha = 0.4) +
  ylim(-100, 150) +
  scale_fill_grey(start = 0.4, end = 0.8, name="Treatment",breaks=c("par", "paruv"), labels=c("P
AR","PAR+UV")) +
  theme_bw() +
  theme(legend.position="none")+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
hp_plot
```

Host protein concentration (ugproteinmgtissu) concentration
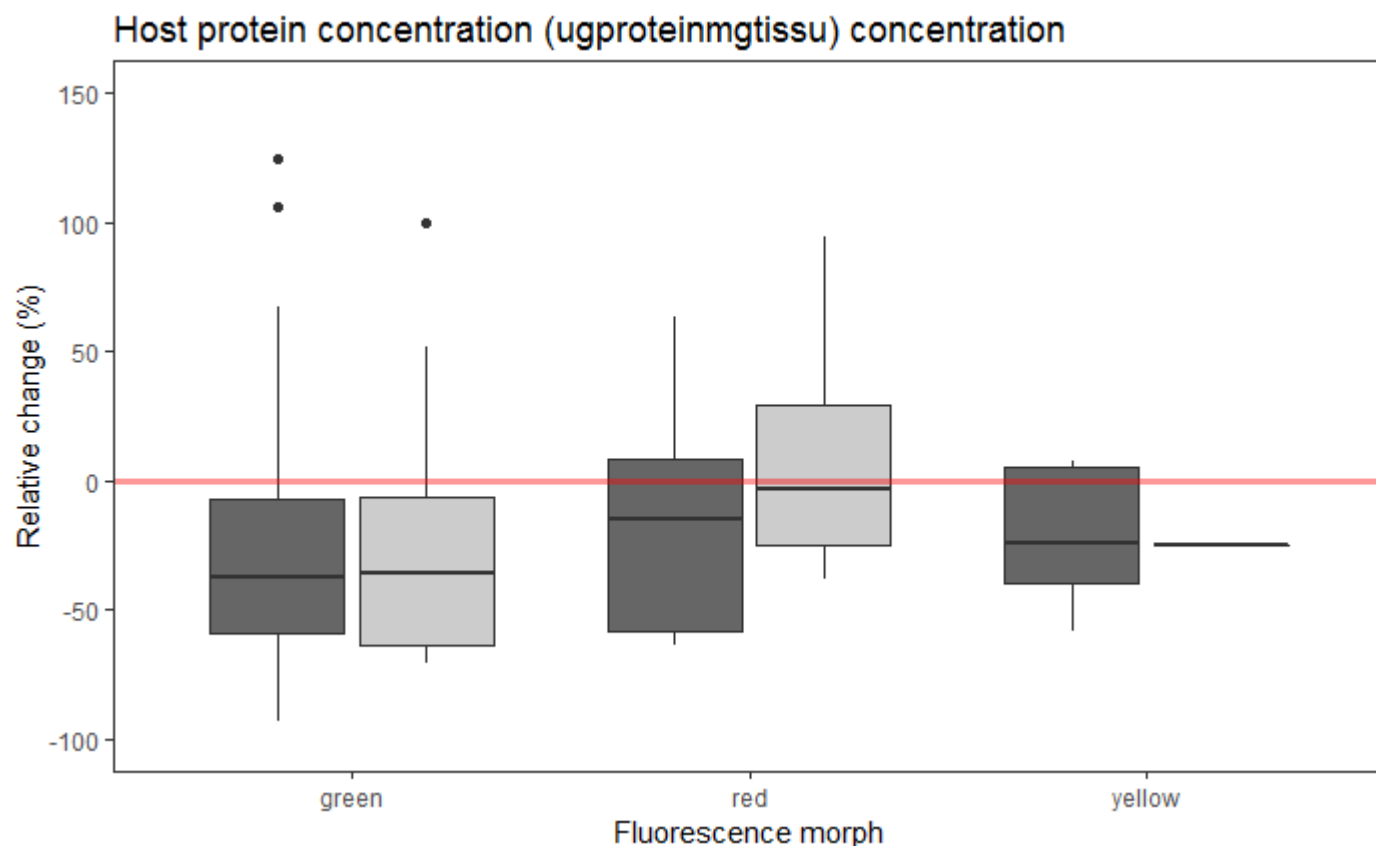

```
maa_plot <- ggplot(data = data_hpmaa, aes(x=morph, y=pmaa)) +
  geom_boxplot(aes(fill=treatment)) +
  labs(title = "MAA concentration (abs320 nmmgtissu)", x="Fluorescence morph", y="Relative chang
e (%)") +
  geom_hline(yintercept=0, color= "red", size = 1.25, alpha = 0.4) +
  ylim(-100, 150) +
  scale_fill_grey(start = 0.4, end = 0.8, name="Treatment",breaks=c("par", "paruv"), labels=c("P
AR","PAR+UV")) +
  theme_bw() +
  theme(legend.position="right")+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())

maa_plot
```

MAA concentration (abs320 nmmgtissu)